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ERRATA.

Page 5, Text-fig. 6, *for* Protopiceoxyon *read* Protopiceoxylon
   „   411, line 34, *for* and winged carpels *read* and wingless carpels
   „   414, „ 22, *for* New Granada *read* Grenada
   ., 464, „ 18, *for* Text-fig. *read* Fig.

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On Protopiceoxylon Johnseni (Schroeter), a Mesozoic Coniferous Wood.[1]

BY

W. N. EDWARDS.

With Plate I and six Figures in the Text.

I N his paper on the fossil woods of King Charles's Land, Gothan (1907, p. 30) deplored the loss of the specimens described by Schroeter (1880) as *Pinus (Larix) Johnseni*, which Gothan definitely states in a later paper (1910, p. 20) should be included in the genus *Protopiceoxylon*. Of the six slides mentioned by Schroeter, three are in the Geological Department of the British Museum, Natural History (V. 13139 to 13141), though there is no record as to how they were obtained. In view of their interest and the progress made in the study of fossil coniferous wood in recent years, it seems worth while to call attention to these specimens and redescribe them for comparison with other species of *Protopiceoxylon* and related genera.

Two of the most important points in connexion with the anatomy of this stem are the pitting of the tracheides and the occurrence of the vertical resin canals. As regards the former, for the most part the bordered pits on the radial walls of the tracheides are in a single row and scattered; occasionally they are in a single row and contiguous, rarely slightly flattened, while infrequently in the early wood there are two rows of pits. These may be opposite or alternate, the latter being the commoner arrangement. Opposition occurs in three or four places, although Schroeter states that he observed it only in one. The alternate pits, even if in contact, are very rarely flattened or compressed, and never quite to the same extent as in the typical araucarioid pitting, or as in *Cedroxylon transiens*, with which Gothan first compared the present species (Gothan, 1907, p. 26). Moreover, a partly araucarioid arrangement of the pits was subsequently described in *Protopiceoxylon exstinctum*, from King Charles's Land and from Spitzbergen

[1] Published by permission of the Trustees of the British Museum.

[Annals of Botany, Vol. XXXIX. No. CLIII. January, 1925.]

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(Gothan, 1910, p. 18).   The other two species of *Protopiceoxylon*, *P. arcti-cum*, Seward (1919, p. 232), from Franz Josef Land, and *P. Edwardsi*, Stopes (1915, p. 81), from the Lower Greensand of Sussex, are not recorded as showing the araucarioid pitting, but additional material of the latter has recently been found with this type of pitting (see below).

As regards the distribution of the resin canals, Gothan in his later paper (1910) abandoned the idea that they might possibly all be traumatic, and therefore associated Schroeter's species with *Protopiceoxylon* rather than with *Cedroxylon*.   With additional material of *P. exstinctum* from Spitz-bergen, he noted a tendency towards a reduction in the number of the resin canals in the outer growth-rings, which is precisely what occurs also in *P. Johnseni*.   In the Spitzbergen material this tendency is accompanied by a change in the character of the outer growth-rings, which have the structure of root wood, that is to say, the late wood forms a band only a few cells broad and is sharply marked off from the earlier, large lumened wood.   In the case of *Protopiceoxylon Johnseni*, the crushing of the cells makes it rather difficult to decide whether or not the later rings are of the root type.   On the whole there is a similar structure throughout the growth-rings, which is well shown in Pl. I, Fig. 2, representing part of the best preserved region in the middle of the wood (about the twentieth ring). Here the first one or two of the early tracheides have very large lumina indeed, giving the wood a striking appearance, and forming a band fairly sharply marked off from the later wood, which is comparatively regularly graduated to the end of the ring.   In the outer rings these larger tracheides have been much crushed, but are still apparently present, and on the whole the same graduation throughout the ring seems to obtain, so that one cannot claim that 'root structure' is well marked.   The specimen therefore sheds very little light on Gothan's suggestion of a correlation between the reduction of the resin canals and the development of 'root structure' in the growth-rings.

In Schroeter's diagrammatic sketch of the transverse section (Schroeter, 1880, Pl. I, Fig. 1) practically no resin canals are shown outside the eighteenth ring, and in his description he states that outside this ring they appear to be quite absent except for a few cavities of uncertain origin in the outermost ring.   There are, however, a few more than he shows which seem to be undoubted canals, and, though usually scattered, there is in ring 25 a regular series of them.   These, however, might possibly be traumatic.   Text-fig. 1 represents a possible canal in ring 26, but those in the external rings are much more doubtful and might be due to decay. The pitted epithelial cells of the resin canals cannot, however, always be seen in the transverse sections.

An interesting point is the presence of thick-walled, pitted elements in the pith (Text-fig. 2).   This feature may be compared with the sclerenchy-

matous plates described by Gothan in the pith of *P. exstinctum* (Gothan, 1910, Pl. II, Fig. 1).

Schroeter states that xylem parenchyma is fairly abundant, occurring particularly in the neighbourhood of the pith, the resin canals, and the autumn wood. His figure (Schroeter, 1880, Pl. I, Fig. 7) was perhaps

TEXT-FIG. 1. *Protopiceoxylon John seni.* Resin canal in ring 26, with decayed remnants of epithelial cells (·). V. 13139.
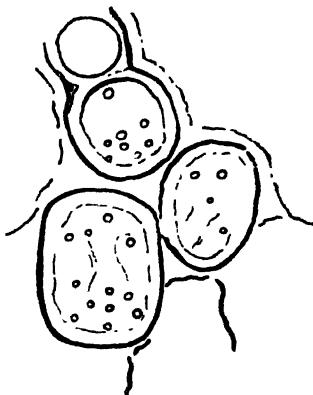
TEXT-FIG. 2. *Protopiceoxylon Johnseni.* Pitted cells in the pith. Transverse section. V. 13139.
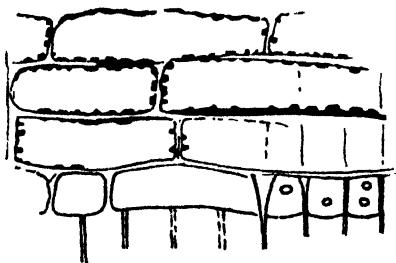
TEXT-FIG. 3. *Protopiceoxylon Johnseni.* 'Abietinean' pitting of ray-cells in radial section. V. 13141.
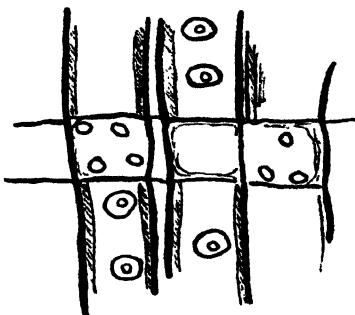
TEXT-FIG. 4 *Protopiceoxylon Johnseni.* Radial section, showing three and four pits in the field. V. 13141.

taken from near the pith; the British Museum radial section does not include the pith and shows only a very little parenchyma apart from that lining the resin canals, though it is undoubtedly present here and there.

For the rest, the main characters are as given by Schroeter. The medullary rays are uniseriate and one to eighteen cells high; there are no horizontal resin canals; abietinean pitting is well marked and can be seen in transverse, radial (Text-fig. 3), and tangential (Pl. I, Fig. 5) sections; the

field contains one to four more or less circular pits which sometimes appear to have a border and in the later wood are somewhat elliptical and oblique. The wood is not sufficiently well preserved to show definitely the presence or absence of bars of Sanio.

The spiral markings mentioned by Schroeter as being seen in tan.ential section are simply the usual decay effects. Text-fig. 5 shows a medullary ray in tangential section with an unusually large cell; this is presumably an abnormality due in some way to decay or disease, and it is an isolated occurrence.

Comparison with Other Species.

Protopiceoxylon Johnseni resembles *P. exstinctum*, but there are certain differences in the character of the growth-rings and in the pitting of the tracheides. A recent examination of Gothan's types in the Stockholm Museum showed that in material of *P. exstinctum* from King Charles's Land the bordered pits are not infrequently in three rows, a point which is not mentioned in Gothan's account. In one case (Slide 2 R) the three rows are opposite and not compressed, and in this slide the araucarioid arrangement is indeed very rare, except for a few 'star-groups' of three pits. In Slide 122 R, on the other hand, an araucarioid arrangement in three rows is not uncommon. The Spitzbergen material of the same species apparently never has the pits in three rows, and in some slides they are always in one row, but the pits are frequently araucarioid, and when in one row are very often contiguous and slightly flattened. In *P. Johnseni*, however, as has been mentioned above, the pits are usually scattered and in a single row.

Of the other two described species of the genus, *P. arcticum*, Seward (1919, p. 232), differs from both in the presence of ray tracheides and the greater depth of the medullary rays; the bordered pits are in two or three opposite rows. *P. Edwardsi*, Stopes, has already been fully compared with *P. exstinctum* (Stopes, 1915, p. 88); it is a very distinct species having abundant small resin canals with few epithelial cells. Early in 1924 a fine block of a stem of *P. Edwardsi*, measuring about 12 cm. in diameter, was found by the Rev. P. de Tennant at Dunnose, Isle of Wight, in Lower Greensand beds, and was presented by him to the British Museum (Nat. Hist.). The tracheidal pitting is better preserved than in the type specimen, and shows bordered pits usually in one row and contiguous, rarely in two rows and compressed (see Text-fig. 6), i.e. rather araucarioid. The resin canals in this specimen are on the average larger than in the type, but are arranged in the same tangential bands. It was suggested by Kräusel (1919, p. 238) that these resin canals in the type specimen were probably traumatic, and that therefore *P. Edwardsi* might be a *Cedroxylon*: the similar arrangement in the Isle of Wight material rather supports Dr. Stopes's opinion that the canals were normal.

Wounding often produces canals in tangential series, and in the case of the Lower Cretaceous *Araucariopitys americana* (a similar type of wood) Jeffrey found such traumatic series in about half the material. The occurrence of rings or series of resin canals in isolated specimens does not necessarily seem to indicate a traumatic origin. However, if the canals in *P. Edwardsi* are eventually proved to be traumatic, the species should be included in *Araucariopitys*.

There are two slides in the Stockholm Museum labelled 'L 34,
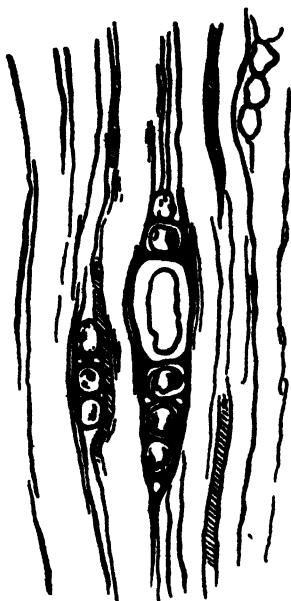
TEXT-FIG. 5. *Protopiceoxylon Johnseni.* Tangential section with abnormal ray-cell. V. 13140.
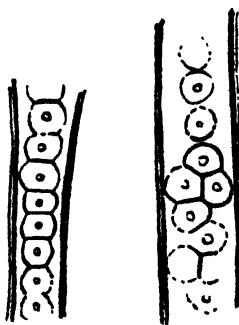
TEXT-FIG. 6. *Protopiceoxyon Edwardsi.* Tracheide pitting in radial section. V. 16418 *b*.

No. 540/23 Norska Exp. 1912, Spetsbergen ', which may be referred to *Protopiceoxylon*. One is a transverse and the other a radial section, and the block is apparently in Christiania Museum. The chief point of interest is the exceptionally large size of the resin canals; otherwise the specimens seem to resemble *P. exstinctum*, but a further investigation would be of interest.

The age of *P. Johnseni* was given by Schroeter as Tertiary, but it is a much older type of wood and is doubtless of the same age as the other woods described by Gothan from King Charles's Land, and considered by him to be either Lower Cretaceous or Upper Jurassic.

SUMMARY AND CONCLUSIONS.

The fossil wood described by Schroeter as *Pinus (Larix) Johnseni* does not resemble in structure any living genus of Conifers, but belongs to a group of Mesozoic woods in which the characters of present-day araucarians and abietineans are combined, but in which the abietinean features seem to predominate.

The fossil woods described under the genera *Araucariopitys*, Jeffrey, *Protocedroxylon*, Gothan, *Protopiceoxylon*, Gothan, and some species of *Cedroxylon* are undoubtedly related, and it is often difficult to draw lines between the genera. The names *Metacedroxylon*, Holden, and *Planoxylon*, Stopes, were admittedly put forward merely because the authors objected to the supposed implications of Gothan's name *Protocedroxylon*; they therefore cannot stand and species included in them must be transferred to *Protocedroxylon*.

On the other hand, if *Araucariopitys* (Jeffrey, 1907) and *Protocedroxylon* (Gothan, 1910) are regarded as indistinguishable, as Kräusel suggests (1919, p. 189), the former name must be adopted on grounds of priority. It would seem useful for the present, however, to keep them separate, and to include in *Araucariopitys* those species with traumatic vertical resin canals, and on the whole with less pronounced araucarioid pitting.

Paracupressinoxylon cedroides, Holden, seems to be an *Araucariopitys*; the presence of xylem parenchyma is not a sufficient ground for instituting a new genus. Other species of this 'genus' appear to be Cupressinoxyla in the wide sense, in so far as they can be identified, and the name *Metacupressinoxylon* recently proposed by Torrey (1923, p. 85) for *Paracupressinoxylon cedroides* is also superfluous.

The few doubtful cavities in *Protocedroxylon Lindleii* (Witham), which might be traumatic resin canals, scarcely warrant placing this species in *Araucariopitys*.

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EXPLANATION OF PLATE I.

Illustrating Mr. W. N. Edwards's paper on *Protopiceoxylon Johnseni*.

All the figures are of *Protopiceoxylon Johnseni* (Schroeter), and the slides are in the Geological Department of the British Museum (Nat. Hist.). The photographs are by Mr. F. W. Edwards.

Fig. 1. Transverse section × 3. V. 13139.

Fig. 2. Transverse section in uncrushed region, showing tracheides with large lumina in early wood. V. 13139.

Fig. 3. Radial longitudinal section, showing partly araucarioid bordered pits. V. 13141.

Fig. 4. Radial longitudinal section through a resin canal, also showing bordered pits, partly in two rows. V. 13141.

Fig. 5. Tangential longitudinal section. V. 13140.

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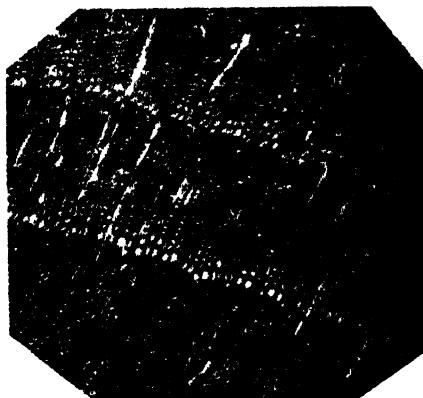
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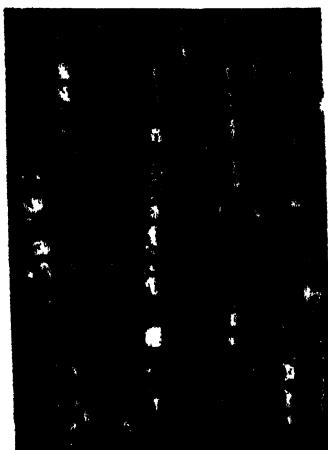
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EDWARDS — PROTOPICEOXYLON.

On the Pneumatophores of Paludal Species of Amoora, Carapa, and Heritiera.

BY

PERCY GROOM, D.Sc., F.R.S.,

AND

S. E. WILSON, M.Sc.

With Plates II and III and ten Figures in the Text.

THE root-system of trees growing in tropical more or less saline littoral swamps varies in form and morphology.

In *Rhizophora* arched or stilt-like roots emitted by the stem descend to the mud as flying buttresses. *Phoenix paludosa*, as described by Gage (1), has, clustered at the base of the stem, remarkable roots which descend through the air into the mud and possess patches of respiratory tissue, comparable with lenticels and with aerenchyma that is produced in place of cork by the phellogen of certain dicotyledons growing in water; these roots in turn bear many small erect lateral roots. In species of *Avicennia* and *Sonneratia* erect conical negatively geotropic lateral roots project above the mud as branches of long horizontal roots. The somewhat similar erect peg-like structures emitted by the horizontal roots of *Carapa moluccensis* are of entirely different morphological nature, and were shown by G. Karsten (4) to be wing-like pneumatophores produced by locally increased activity of the cambium. *Carapa obovata* possesses no such erect growths, but its creeping roots are band-like with the upper edge knife-like and projecting above the mud, according to A. F. W. Schimper (8, 9). The knee-like pneumatophores of *Bruguiera eriopetala* are parts of the long horizontal roots, whereas in *Lumnitzera racemosa* they are portions of the lateral roots given off by the long creeping roots.

Although considerable attention was devoted by K. Goebel (2, 3), G. Karsten (4), H. Schenck (6, 7), and others to the anatomy (and, in Karsten's case, to the respiration) of the tissue outside the wood of these pneumatophores, scant attention has been paid to the structure, contents, and physiology of the wood. Yet, on the one hand, the pith-like

wood in roots of certain aquatic Leguminosae (*Aeschynomene* and others) is recognized as respiratory in function, and, on the other hand, A. F. W. Schimper pointed out that the tissue outside the wood of the knee-like roots of *Bruguiera caryophyllata* peels off.

The present paper deals exclusively with pneumatophores morphologically homologous with those of *Carapa moluccensis*, and particularly with the structure of their wood, and is partly designed to stimulate further research in the tropics on the physiology of that wood.

Amoora cucullata, Roxb.

This meliaceous tree grows in swamp forests, for instance in the Sundribuns (India), whence the material used in this investigation was obtained. Here its root-system includes long horizontal roots[1] which bear on the upper surface erect outgrowths shaped like long flattened pegs (Pl. II, Fig. 1) which emerge from the mud. As each horizontal root is roughly elliptical in cross-section with the long axis of the ellipse vertical, it together with the outgrowths resembles a gigantic comb whose teeth are uneven in length and in distances apart. The erect peg-like structures are termed by systematists 'root-suckers', but as they are neither roots nor stems, they will in this paper be termed 'pneumatophores'. Occasionally they bear on their sides slender cylindrical organs that are true roots.

A. HORIZONTAL ROOT.

The horizontal roots are epinastic and dorsiventral, as is shown by the much greater width of the growth-rings (cp. annual rings) of the wood on the upper than on the lower side, and the consequent excentricity of the organic centre as seen in transverse section (Text-fig. 1 *a*).

The growth-rings in the specimens examined were continuous round the organic centre, and were $8\frac{3}{4}$ in number in the root examined. The excentricity of structure of the wood and the change in shape of the successive growth-rings cause the medullary rays to be curved in transverse section (except in the median vertical plane): in the succeeding description of the horizontal root, the term 'radial' denotes directions parallel to the curved rays as seen in transverse section.

(a) The Wood.

The wood consists mainly of wood-fibres, among which are interspersed vessels, perhaps a few tracheides, wood-parenchyma, and numerous medullary rays.

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[1] The material available to us provided no evidence that these are roots, and not stems, but for the sake of brevity they will be described as roots.

The tracheae are solitary or ranged in small, usually radial groups ; the larger ones are richly pitted, tend to be isodiametric in transverse section, and have large simple nearly transverse openings, although frequently a segment has a long tail-like hollow continuation on one side. In a group of vessels some are narrower, radially flattened, and occasionally have oblique openings (it is possible that occasionally these may be represented by tracheides). Except in the outermost growth-ring, the apertures of many of the wide vessels, and of some of the narrow ones, were plugged by single cushion-shaped masses recalling the calli of sieve-tubes. This fact
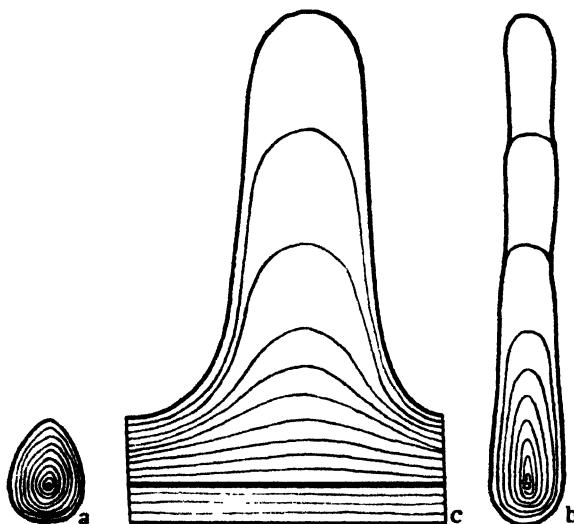
TEXT-FIG. 1. *Amoora* : diagrams of the wood : *a*, transverse section of a horizontal root ; *b*, transverse section of a horizontal root also passing through the middle plane of a pneumatophore; *c*, vertical radial longitudinal section of a horizontal root also passing through the middle plane of a pneumatophore.

suggests that the water-conducting powers and activities of many of the vessels are lessened quite early in their lives, but injections of eosin solutions proved that water travelled readily in the vessels even under a pressure of one atmosphere.

The very abundant *wood-fibres* are typically meliaceous in structure in being septate, not having narrow lumina, and in possessing simple pits (which are slit-like and ascend in a spiral). In transverse section the fibres are largely ranged in radial series, so that the wood shows a superficial resemblance to coniferous wood, and this likeness is enhanced by the thinner walls and wider lumina of the fibres at the inner part of the growth-ring, and also by the radial flattening of the fibres near the outer boundary of the growth-ring. The fibres having thicker walls owed their increased thickness of wall to a larger deposit of an inner layer that was less lignified (and

gave a green colour with iodine and Vétillart's dilute glycerine-sulphuric acid). In the outermost growth-ring the fibres contained protoplasm ; elsewhere no solid contents were visible.   The ends of the fibres are pointed, and often assume the form of unilateral tail-like processes.   This last character recurs in the wood-parenchyma, so that transitional types between these two tissue elements occur.

The *wood-parenchyma* is ranged in the normal longitudinal series : its cells, while showing considerable variation in form, are mainly brick-shaped, shorter and wider than the segments of the wood-fibre, and their walls bear more numerous and larger pits, yet there are longer parenchyma-cells transitional towards wood-fibres.   Parenchyma of narrower form, with occasional medullary ray-cells or wood-fibres, constitute the main circum-vasal tissue.   In the outermost wood these cells contained protoplasm, in the innermost growth-ring some contained starch, but elsewhere their solid contents were limited to small quantities of substances of unknown nature.

The *intercellular spaces* are not well developed as seen in transverse section, but are less indistinct in radial section at the angles of the medullary ray-cells.

The *medullary rays* are very numerous, mostly one cell, occasionally two cells, thick, and in relation to their thickness are unusually high (up to eighty cells).   The cells, narrow tangentially, vary in shape in radial section from a square to an upright or prostrate rectangle.   The erect cells occur at the margins, but also in the interior of the ray, and their presence near wood-vessels often causes rays in tangential section to simulate wood-parenchyma.   In the outer wood the ray-cells contained protoplasm ; and elsewhere they had more abundant contents (starch and substances of unknown nature) than had any other constituents of the wood.

Regarding the *physiological anatomy* of the wood of the root, the first feature standing out is the relatively feeble development of the water-conducting tracheal elements, and the early appearance of plugs that appear to block the apertures of many of the vessels.   The second feature is the abundance of the elements containing no solid contents ; namely, not only the tracheal constituents but also most of the fibres.   This poverty in contents may be partly, but only partly, due in the case of the fibres to the action of Fungi which were growing in the wood.   The third feature of interest is the small development of the intercellular system.   These three sets of facts suggest that possibly vessels and fibres were largely air-containing in the living tree and acted as reservoirs and conductors of air.

(b) Secondary Cortex.

The tissue outside the cambium was so badly preserved and permeated by fungal hyphae that the normal structure, especially as regards the inter-

cellular spaces, could not be observed. Cork-cells, lenticels, longitudinal rows of crystal-containing stone-cells with lignified walls, and medullary rays were noted.

B. PNEUMATOPHORE.

(a) *Wood.*

The origin and development were revealed by the structure of fully developed organs and of certain pneumatophores whose growth had been prematurely arrested. The pneumatophore arises in the following manner. At certain isolated places along the length of the young horizontal root the activity of the cambium is greatly increased on the upper half, so that there arises at each place a low fin-like vertical wing elongated in the direction of the root-axis. This stage is approximately represented by the low arrested pneumatophore (*a*) in Text-fig. 2. Further growth takes place solely through the activity of the cambium, and causes the low wing to develop into an erect peg-like pneumatophore; this then is an organ that grows in height (length) by means of an apical cambium, which, however, extends down the flanks into the root and is continuous with the cambium of this.

As the low rudiment gradually gives way to the well-developed pneumatophore (often 2 ft. in height), striking changes take place in the orientation of the cells and vessels.

A *vertical radial longitudinal section* of the horizontal root taken so as to pass through the (organic) median plane of the pneumatophore shows the growth-rings continuing from the former up into the latter, the fibres and vessels cut longitudinally radially and running parallel to the outlines of the growth-rings, and the medullary rays likewise cut radially and crossing the growth-rings at right angles (Text-figs. 1, *c.* and 3). Tracing the growth-rings from the root to the pneumatophore, beginning at the inner rings and working outwards, the outlines of these (and the course of the vessels and fibres), which are straight and horizontal in the normal part of the root, become increasingly bowed upwards so as ultimately to form a series of superposed arches of rapidly increased height but slowly increased width : thus at the sides of the well-developed organ the vessels, fibres, and outlines of annual rings are erect and at right angles to their direction in the horizontal root. The medullary rays in the middle vertical line of this section are straight and vertical in root and pneumatophore, but on either side of this line they diverge and curve outwards (being anticlinal to the periclinal growth-rings and vessels), until their outer extremities at the vertical flanks of the pneumatophore are horizontal (Text-fig. 3 ; Pl. II, Fig. 2).

For convenience of description, the plane of section just described may be regarded as dividing the pneumatophore into right and left halves.

Viewing either the right or left outer surface of the intact pneumatophore, the arch-like basis of the construction is to some extent indicated by the cork (Pl. II, Fig. 1). If the tissue outside the wood be removed, the right or left face of the wood shows a series of arches (Text-fig. 2, lower part).

Thus the whole pneumatophore may be regarded as in essence a somewhat laterally compressed cone consisting of a number of plane and more or less curved sheets parallel with the long axis of the root, and each consisting of a series of superposed arches, the height and steepness of the arches decreasing as the planes are nearer to the right and left faces.

The course and continuity of the vessels in root and pneumatophore were also demonstrated by eosin dissolved in water that was forced in at the ends of cut surfaces, transverse to the course of the vessels, by a pressure of one atmosphere (Text-fig. 2). The eosin solution travelled rapidly along the injected vessels.

A *transverse section* of the horizontal root taken so as to pass through the (organic) centre of the pneumatophore only to some extent presents the same appearance as if the root possessed a long continuous erect wing in the middle of its upper face. At the base the growth-rings continue from the root to the pneumatophore, and several are seen enveloping the older ones above and on the sides, but here in this plane growth in thickness (as opposed to height) is increasingly restricted with age (Text-fig. 1, *b*). Higher up, the youngest growth-rings form merely a single series of superposed tall concavo-convex cushions of tissue, each representing a single period of growth (Text-fig. 4). In the inmost proximal growth-ring where several occur, and in the most distal single one of superposed growth-rings, all the constituents (vessels, fibres, wood-parenchyma, and medullary rays) are transversely cut; in the middle vertical line the medullary rays are straight and vertical, and continuous from root into pneumatophore, but on either side of this line they curve outwards and preserve their directions perpendicular to the outline of the growth-ring, with a result that at the base of the latter the outer extremities of the rays are horizontal or nearly so (Text-fig. 3).

The section just described is a transverse section of the horizontal root, but would naturally be regarded as a longitudinal section of the pneumatophore; in order to emphasize the true morphological nature of the pneumatophore, and to avoid ambiguity, this vertical plane and those parallel to it are hereafter termed transverse planes. The transverse plane passing through the organic centre of the pneumatophore may be regarded as dividing this into acroscopic and basiscopic halves.

During the growth in thickness of the proximal region of the pneumatophore, the vessels, fibres, and wood-parenchyma deposited outside pre-existing tissue gradually assume a vertical direction. This is attained
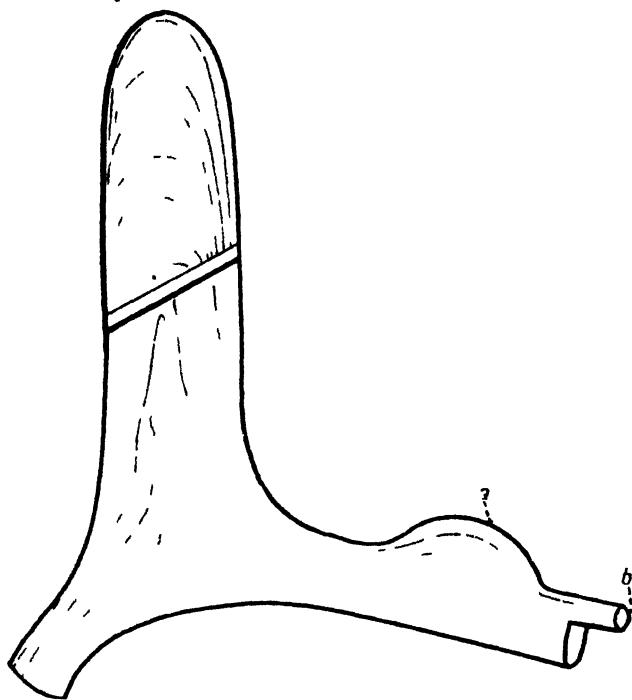
TEXT-FIG. 2. *Imoora* diagram of the wood of a horizontal root, a normal pneumatophore, and an arrested one at *a*, injected with eosin from end *b*. The lower part of the diagram represents the surface of the outermost wood, the upper part of the normal pneumatophore represents the internal wood in the middle plane
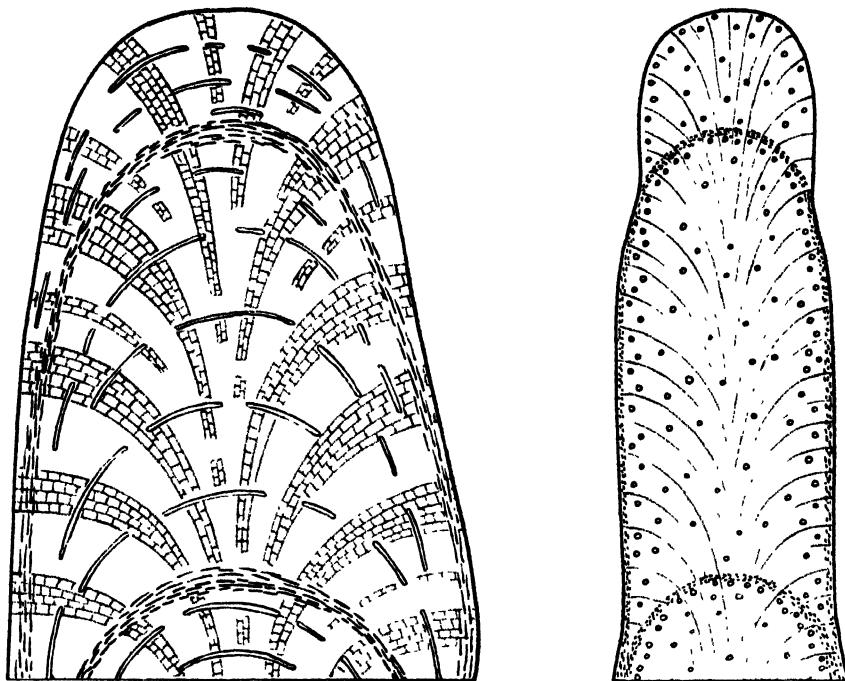
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TEXT-FIGS. 3 and 4. *Amoora* : diagrams of the wood of the top of a well-developed pneumato-phore, showing one locally complete growth-ring, an incomplete terminal growth-ring, the top portion of a third ring, and the boundaries of these, also showing wood-vessels and medullary rays : in 3 all the constituents are seen in radial longitudinal section, as the section is cut in the vertical median radial plane (of the horizontal root) : in 4 all the constituents are seen in cross-section, as the section is a vertical one cut through the middle of the pneumatophore in a plane transverse to the horizontal root.
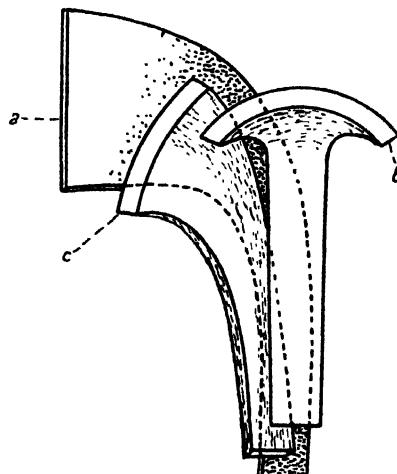
TEXT-FIG. 5. *Amoora* : diagrams of three medullary rays belonging to a vertical portion of a pneumatophore; *a* in the middle line of the acroscopic or basiscopic face; *b* in the middle line of the right or left hand face; *c* in the line between the two preceding.
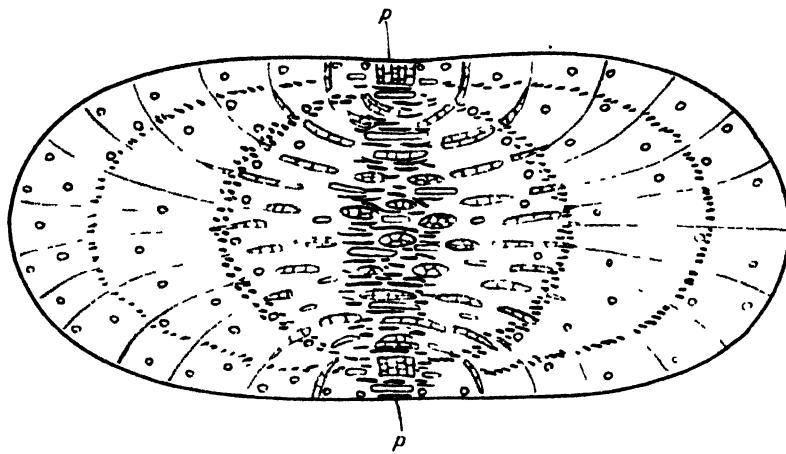
TEXT-FIG. 6. *Amoora* : diagram of the wood as seen in a cross-section of a pneumato-phore where three growth-rings are visible : *p, p* represent the arch-patches; at the central point of the short axis *p* to *p* the medullary rays and vessels are cut in longitudinal tangential section, but travelling out towards *p*, the direction of the section gradually changes to longitudinal radial; travelling from the central point outwards in other directions, the direction of the section changes to transverse, so that in the two outer growth-rings the wood is seen in transverse section excepting at the arch-patches.
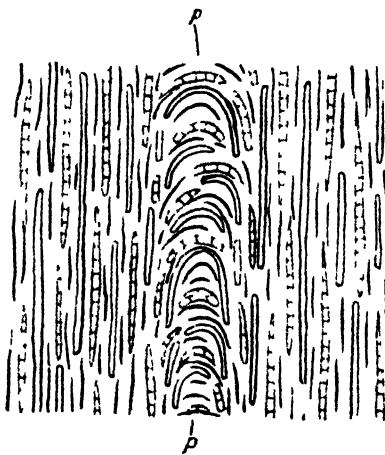
TEXT-FIG. 7. *Amoora* : diagram of wood cut in a vertical tangential plane parallel to the median vertical radial plane of the horizontal root, and passing through the arch-patch *p p* : the constituents are approximately cut in longitudinal tangential direction, but the vessels in the central line *p p* are approximately horizontal and the rays are prostrate, whereas outside the arch-patch area the vessels are vertical and the rays upright.
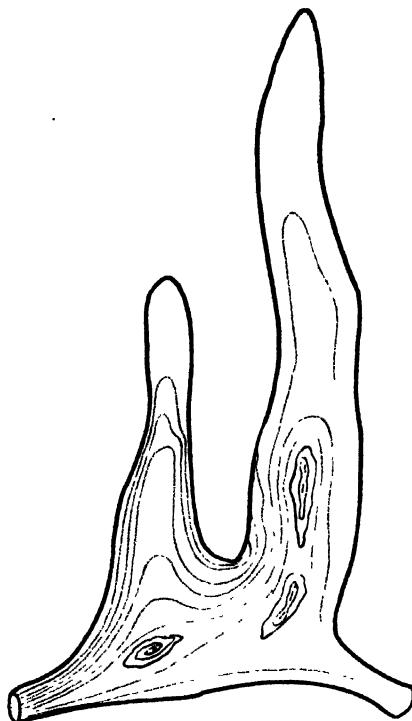
TEXT-FIG. 8. *Carapa*. Two partially fused pneumatophores injected from the left-hand cut surface of the horizontal root with eosin; also showing three superficial islands of wood-tissue where the arch-structure is replaced by closed loops.

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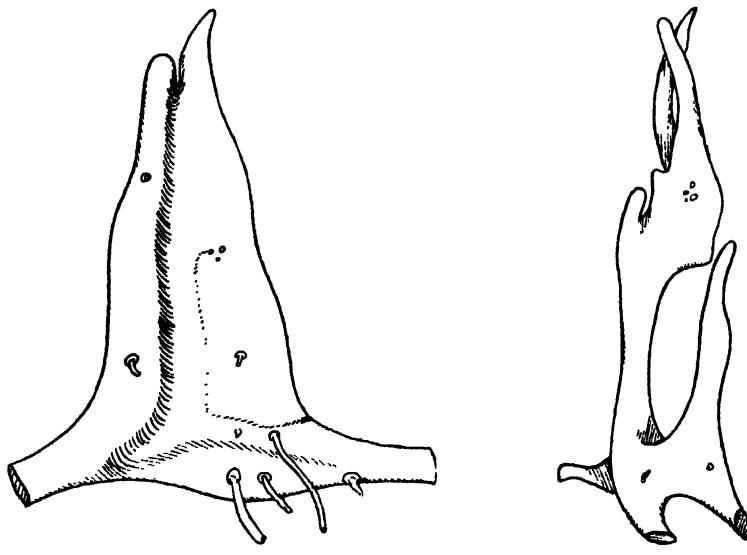
TEXT-FIG. 9. Broad two-tipped pneumatophore with lateral roots and portion of the horizontal root. Reduced. (Dimensions of the original pneumatophore 50 cm. high, 20 cm. at base in direction of the long axis of the horizontal root, and 1·5-2 cm. in a direction transverse to the latter.

TEXT-FIG. 10. A pneumatophore doubly forked at the end. Reduced.

earliest by constituents in the median lines of the basiscopic and acroscopic faces (i.e. in the median radial plane), and the change gradually progresses round the base of the pneumatophore towards the (organic) middle lines of the right and left faces, until the arch-like construction originally seen in these faces is obliterated or preserved only in miniature at the actual base. Thus a vertical section in the transverse plane (of the root) passing through the organic centre of the pneumatophore would (if it were really possible to cut such a section) still show, where the remnants of the arch-construction persisted, all the vessels and fibres cut transversely, whereas a vertical section in a parallel plane close in front or behind this would show the inmost growth-ring cut nearly in cross-section, but the outermost growth-ring cut in longitudinal radial section (cp. Text-fig. 6).

In passing from the inside to the outside of the more proximal parts of the pneumatophore, the change in direction of the vessels and fibres is associated with a remarkable twisting of the medullary rays. In order to explain this in detail, it is necessary to define terms as applied to medullary rays. The *height* of a ray is its linear dimension as measured in the direction of the longitudinal course of the contiguous vessels or fibres. The *thickness* is the maximum dimension perpendicular to the height as seen in tangential section. On a vertical organ a ray may be termed *upright* or *erect* if its height and sides are vertical, *prostrate* if these be horizontal, and *oblique* if transitional between these two extremes. In the inmost growth-ring a surface view of the wood of the middle lines of the basiscopic and acroscopic faces of the pneumatophore, where this is erect, shows the vessels and fibres vertical, and consequently the medullary rays (in tangential view) as vertical lines of parenchyma (Text-fig. 5, *a*), but a surface view of the middle vertical lines of the right and left faces shows the vessels and fibres forming the tops of arches and the medullary rays (in tangential view) prostrate (horizontal) and curved parallel to the vessels and fibres (Text-fig. 5, *b*). Surface views intermediate between these two show the vessels, fibres, and heights of the rays oblique in direction (Text-fig. 5, *c*). In passing from the inside to the outside of this growth-ring, all the rays ascend and bend outwards. Outside this ring, when once the rays in the median vertical plane (of the root) have attained their horizontal direction they continue in their straight course, so that in this plane the rays merely bend, so to speak, on their edges. But in the transverse plane (of the main root) outside the inmost growth-rings of the pneumatophore, as the direction of the vessels and fibres gradually becomes more erect, the rays as seen in tangential view twist from a horizontal (prostrate) pose to an oblique, and finally an erect pose where the arch-structure disappears. Between these two planes the rays in passing outwards execute twists, the magnitude of which is proportionate to their angular distance from the median longitudinal vertical plane of the main root.

A *cross-section* of the pneumatophore taken near the base of either

a solitary distal or an inmost proximal growth-ring is roughly elliptical in outline, with the long axis of the ellipse parallel to the length of the horizontal root (Text-fig. 6; Pl. II, Fig. 5). The middle part of this long axis shows the vessels, fibres, and medullary rays cut tangentially, as the section here passes through the top of an arch of fibres, and the vessels and heights of the rays are parallel to the axis in question. Moving outwards along this axis, the course of the vessels and fibres becomes oblique, until near and at the periphery these and the medullary rays are cut transversely. On the short axis of the ellipse (transverse to the horizontal root) the vessels and fibres are everywhere seen in longitudinal view with their direction parallel to the long axis of the ellipse, but in passing from the centre to the periphery the view of the medullary rays gradually changes from tangential to radial. This section shows the outermost wood cut transversely, except at two places at the ends of the short axis of the ellipse and where two patches of tissue are the tops of arches of vessels and fibres, which are seen in radial longitudinal section : these two patches may be termed the arch-patches (cp. Text-fig. 7). Cross-sections of the pneumatophore where several growth-rings occur show all the outer rings cut transversely except at the two arch-patches, which assume the form of triangles with the apex outwards (Pl. II, Fig. 5, *p*), as the adjoining tissue cut transversely gradually encroaches upon them, until at a certain height up the pneumatophore (where five growth-rings were visible in the specimen examined) the last traces of the arched structure disappear and the whole of the external wood is cut transversely, but lower down this pneumatophore (where six growth-rings were visible) arches still persisted, and were visible in the cross-section as triangles with their fine apices reaching the surface.

Cross-sections of pneumatophores showed that by enhanced growth in thickness in the proximal parts in the direction of the length of the root the original elliptical outline became replaced by a more oblong shape, and that the outer acroscopic and basiscopic parts had become thicker than the more central part connecting them ; moreover, the original inmost growth-ring had become excentric, because of the unequal thickening in the longitudinal plane of the horizontal root of the acroscopic and basiscopic halves. Finally, these sections showed that the arch-patches, which should have been opposite to the ends of the short axis of the original elliptical growth-ring, had been displaced by uneven growth, with the consequence that the pneumatophore had in truth no plane of symmetry.

Ignoring this last minor disturbance of growth, the wood of the pneumatophore begins existence as an isobilateral structure with a vertical plane of symmetry and the longitudinal axis horizontal; but finally in portions of its outermost proximal parts it acquires radial symmetry, whereas its distal portion remains isobilateral, and the longitudinal axis is erect. In design and morphology then it is a kind of semi-symmetrical burr.

In order to emphasize the symmetrical designs displayed by the pneumatophore, certain irregularities of growth exhibited by individual specimens have not been mentioned. In one specimen the outermost wood showed on the face of the base and 2 inches below the tip the replacement of the arched structure by closed oval islands of wood recalling the ' eyes ' of a burr : these arise when in the organically middle line of the right or left faces of the pneumatophore the fibres and vessels assume a more erect pose earlier than do those immediately above them (cp. also Text-fig. 8).

Histology.

Histologically the wood generally agrees with that of the horizontal root, but presents certain deviations.

On the flanks, where the vessels are running straight and vertically, there is no marked difference as regards relative number or distribution of the *vessels*, but in the upper distal thicker part of the growth-ring the increased width is largely due to increased number of the fibres, so that there is a relative decrease in the number of vessels : probably the vessels present in the latter parts of the growth-ring are mere continuations of those present in the thinner more proximal parts of the growth-rings. The callus-like *plugs* appearing to block the apertures of the wood-vessels occur even in the outermost growth-ring, and seem to occur indifferently on the upper and lower face of the aperture in the acroscopic and basiscopic halves of the pneumatophore : thus the water-conducting system of tracheae appears to be relatively reduced in quantity and more obstructed than in the horizontal root.

Starch was more abundant in the parenchyma and medullary rays of all growth-rings, so that the pneumatophore to some extent represents a starch-reservoir.

The *medullary rays*, which are mainly uniseriate and tall where the vessels run straight in the root also in the pneumatophore, become shallower and plumper in the latter where the vessels are curved. At least in some cases the thinning of the rays in their outward passage is caused by a single ray fraying out into several uniseriate ones.

Lignification. As judged by staining in phloroglucin and hydrochloric acid, the walls of all the constituents of the wood are well lignified.

(b) Secondary Cortex.

The tissue outside the wood was not sufficiently preserved for critical examination.

The same constituents as occur in the root were recognized, but the thin-walled cork had in addition within it several layers of thick-walled cork-

cells, which were lacking within a lenticel, and the sclerenchyma was more abundant. The utility of the greater development of the sclerenchyma in cork and within is suggested by the fact that the pneumatophores are liable to mechanical injury by animals and particles of earth (indeed the pneumatophore examined showed that early in life the tips had been wounded). In the secondary phloem, in cross-section, the medullary rays rich in tannin bodies together with tangential lines of parenchyma rich in tannin bodies form a network with rectangular meshes in which is lodged the remaining tissue.

LATERAL ROOTS.

Slender lateral roots are occasionally emitted from the flanks of the pneumatophore. One available for examination was sufficiently preserved to show that it was radially symmetrical, had one growth-ring of wood in which the vessels were relatively more numerous than in the pneumatophore and horizontal root, and that in the delicate secondary cortex parenchyma and rows of crystal cells were present.

Carapa moluccensis, var. gangetica.

G. Karsten (4) described *C. moluccensis* as possessing long horizontal roots that bear on the upper side characteristic horn-like erect pneumatophores. As these latter are laterally compressed, this system of roots and pneumatophores agrees in external form with that of *Amoora*.

It may be added that the pneumatophores often bear thin cylindrical roots, and that sometimes two pneumatophores are attached close together with a common base produced in the same manner as the tissue common to the double leader of a tree (Text-fig. 8).

Although Karsten described in some detail the anatomy of the tissue of the pneumatophore outside the wood, and in particular the large intercellular aerating system, he scarcely dealt with the structure of the wood, but confined himself to provision of a diagram of the wood of a young pneumatophore cut in a median vertical radial plane.

Wood of the Pneumatophore.

In origin, development, and general type of construction, the wood of the pneumatophore of this species (*gangetica*) agrees with that of *Amoora cucullata*, so that it will here suffice to describe the former very briefly and show how it differs anatomically from the latter.

The growth of the pneumatophore of *Carapa* examined was more irregular than that of *Amoora* (cp. Pl. III, Fig. 6). While the general arch-like construction of the wood is revealed by mere inspection by sections, and by injections of eosin solution, irregularities of growth cause the arches (for instance, as seen in surface view of the right and left faces) to be repre-

sented in places by closed loops (Text-fig. 8) comparable with the ' eyes ' of burr-wood.

The seeming presence of *growth-rings* is at least partly spurious and due to metatracheal bands of parenchyma that run generally more or less parallel to the contour of the pneumatophore as seen in longitudinal radial and cross sections (but see Pl. III, Fig. 6), in both of which bands may be seen fraying out into finer ones, which either end blindly or link up with others. If true growth-rings be present we failed to identify them.

The inner and terminal (distal) wood consists mainly of fibres, medullary rays, and some wood-parenchyma : the outer wood, especially on the right and left flanks, includes relatively more numerous and larger vessels.

The *wood-vessels* are scattered, and occur even in the metatracheal bands of parenchyma ; they are solitary or in usually radial groups of 2–4 ; for the most part they were devoid of contents, so that their oval apertures were unobstructed, but here and there they contained solid substance in quantity. The wood was easily injected with eosin solution.

The *wood-parenchyma* is more abundant than in *Amoora*, for the long metatracheal sheets are supplemented by circumvasal (paratracheal) and other parenchyma. In the metatracheal sheets the cells are in radial series and 4-sided in cross-section. In longitudinal section they vary considerably in length and often show a tiered arrangement radially (Pl. III, Fig. 7). As much wood-parenchyma and many of the cells of the medullary rays contained considerable starch, the pneumatophore can represent no mean starch-reservoir. Many wood-parenchyma cells and ray cells contained globules or masses of a substance containing tannin. Finally a number of cells of the wood-parenchyma and rays showed no contents.

The *wood-fibres* are septate, and many have wide lumina and moderately thick walls ; others had smaller lumina and thicker walls, the pits being simple. Most of the fibres were devoid of solid contents.

The *medullary rays* are numerous, and thicker than those of *Amoora*, being often triseriate (Pl. III, Fig. 9) where the adjoining vessels are straight, but shallower and often quadriseriate where the vessels are curved (Pl. III, Fig. 8). In the latter regions, in a tangential section parallel to the long axis of the root, the rays may form at least half of the tissue. The cells vary in form, being prostrate, brick-shaped, cubical (small, or large and occupying the whole thickness of a biseriate ray), or tall, erect, and tapering at the upper and lower margins of the ray. The presence of the last-mentioned ray-cells and of tiered wood-parenchyma rendered it impossible in every section to distinguish between ray-cells and wood-parenchyma, especially where two rays were superposed.

Lignification. As judged by depth of staining in phloroglucin and hydrochloric acid, the walls of the vessels and fibres are well lignified, whereas those of the wood-parenchyma and medullary rays are feebly so.

C 2

The *intercellular spaces* are not markedly developed.

' *Resin-glands* ' are visible in cross-sections of the wood as arcs of disorganized tissue including a large amount of so-called resin : the gland continues for some distance in a longitudinal direction (Pl. III, Figs. 7, 9). A description of these (and other meliaceous) internal excretory glands will be given in a subsequent paper, but here it may be stated that they frequently, though not invariably, arise in the thick metatracheal belts of parenchyma.

Heritiera minor, Roxb.

Heritiera minor, ' Sundri ', gives the name to the type of swamp forest in the Sundribuns, whose flora and vegetation have been so fully described and discussed by D. Prain (5).

Although this plant belongs to the Sterculiaceae, its erect peg-like pneumatophores agree in origin, development, and general construction with those of the two Meliaceae previously described. Their morphological nature as localized wings of the horizontal root is emphasized by D. Prain's observations, that these laterally compressed outgrowths are wider in the direction of the longitudinal axis of the horizontal root near the main trunk than at more distal places, and that they often coalesce with and become parts of the buttresses at the base of the trunk. A specimen in the Botany Department of the British Museum (Natural History) strongly suggested that the peg-like pneumatophores can fuse. The pneumatophore (Text-fig. 9) in question has the form of a flattened triangular plate terminating in two rather short peg-like processes ; the horizontal root and the pneumatophore bore numerous downwardly directed cylindrical rootlets. But another specimen (Text-fig. 10) in the same Museum showed that a pneumatophore can bifurcate more than once (probably thanks to apical injury).

D. Prain made the interesting observation that the pneumatophores arise only where the horizontal root branches.

The pneumatophore that we microscopically examined was more irregular in structure than that of *Amoora*, for in places the arch-like pattern of construction was locally replaced by a burr-like tangle of tissues (Pl. III, Fig. 10).

WOOD OF THE PNEUMATOPHORE.

Growth-rings (Pl. III, Fig. 11) are marked by the concentric bands of parenchyma, whose cells are much smaller in transverse section than those of the general wood-parenchyma, are radially compressed, and tend to be ranged in radial series, though the bands vary from one to four cells in thickness. Moreover, the part of the growth-ring lying immediately radially within such a band is richer in wood-fibres than elsewhere, and the fibres here tend to be ranged in more closely set concentric series. Finally, the cells of the parenchyma bands in the material examined contained more starch than did the general parenchyma of the same growth-ring.

The wood consists of vessels, very abundant wood-parenchyma, characteristic wood-fibres, and abundant medullary rays.

The *vessels* are scanty in the central wood, and by no means numerous in the wood on the flanks. They are solitary or in groups, usually radial series including up to five vessels.

The *wood-parenchyma* occurs in three extreme forms : first, the general parenchyma, consisting of large thin-walled cells with well-pitted walls secondly, cells in contact with the vessels that are narrower and very richly pitted ; thirdly, brick-shaped radially compressed cells at the boundary of the growth-rings.

In the older wood the general parenchyma cells were largely empty, although the medullary rays here contained much starch, but in the outermost wood the general parenchyma was starchy.

In transverse section the *wood-fibres* are either solitary, in nests, or in tangential series : they do not run straight in a longitudinal direction, but are meandering in their course, and link with one another to form a network that gives a mechanical strength to the wood. which is so largely composed of weak thin-walled parenchyma (wood-parenchyma and rays). The fibres are irregular in form, widening or narrowing locally near their ends or elsewhere, and bulging into depressions formed at the juncture of two parenchyma-cells. They are narrow, thick-walled, with rather small lumina, and in transverse section vary from triangular to polygonal in shape.

The *medullary rays*, together with wood-parenchyma, are seen in tangential sections to form the main mass of the wood. In tangential sections (for instance, taken in the transverse plane of the horizontal root), where the course of the vessels is straight, the rays range from linear to fusiform in shape, and from 1-seriate to 5-seriate ; internal to the youngest wood their cells stood out from the wood-parenchyma by reason of richer contents, and they could thus be distinguished from the latter even when they were upright and tall. In the same section the wood-parenchyma separating two superposed medullary rays shows a tiered arrangement. In a radial section of the wood where the vessels are straight, the cells in the interior of the ray are prostrate and radially elongated, but at the upper and lower margins the ray-cells are deeper, oblong and prostrate, square, and even oblong and erect ; and where rays are superposed, these marginal cells are succeeded above or below by more or less elongated or short wood-parenchyma cells arranged in tiers : consequently in such a section it is impossible to define with certainty the boundaries of the rays and wood-parenchyma.

Where the course of the vessels and fibres is curvilinear, a tangential section of the pneumatophore taken in the vertical longitudinal plane of the main root may show that growth can be so irregular that the arch-design is replaced by burr-like irregularity of construction (Pl. III, Fig. 10). In

such a section the vessels run in irregular indented curves ; the rays of the wood-parenchyma and fibres form closed and open loops ; while the thicker medullary rays (tangentially cut) are plump and shallow, varying in form from slightly curved fusiform to thick crescent-shape, and to utterly irregular shapes ; moreover, the rays are largely oblique or prostrate in pose.   In some of these tangential sections this irregularity of structure was enhanced by the occurrence of relatively large circular islands of rather thick-walled parenchyma cut transversely.

ROOTLETS OF THE PNEUMATOPHORES.

The pneumatophore emits rootlets, which themselves may be branched. The constituents of the wood agree with those of the pneumatophore, but are so arranged as to render the rootlet radially symmetrical.   The growth-rings (five in number in the sample examined) are marked by bands of radially compressed parenchyma which had richer contents than had the remaining abundant parenchyma.   In the outer growth-rings the vessels are much more numerous and larger than in the more central rings.   The fibres are similar to those of the pneumatophores, and similarly distributed in the parenchyma.

SUMMARY.

The erect peg-like pneumatophores of two meliaceous species, *Amoora cucullata* and *Carapa moluccensis* var. *gangetica*, and of the sterculiaceous *Heritiera minor*, are arranged in a series along the middle of the upper surface of the long horizontal epinastic axes (roots ?) that run beneath the surface of the mud of Indian saline swamp forests.   In origin and general development they agree, as each is a laterally compressed horn-like dorsal wing produced by localized activity of the cambium, and continuing to grow in height and thickness by means of a cambium continuous over the wood. Some pneumatophores are forked, either low down or near the tip.   In some cases (*Carapa*) this type of construction is induced by fusion of two juxtaposed pneumatophores, and is akin to the merging of a pneumatophore with the low plank-buttresses at the base of the trunk (*Heritiera*) ; in other cases the forking is due to bifurcation at the tip of a growing pneumatophore (*Heritiera*).

The pneumatophores of all three species investigated may bear thin cylindrical organs, which in the two cases investigated (*Amoora, Heritiera*) are radially symmetrical rootlets.

The horizontal roots (axes) are dorsiventral epinastic.

The wood of the pneumatophores in main design initially consists of vessels, fibres, axial parenchyma ranged in series of superposed (periclinal) arches whose planes or curved surfaces are in the direction of the longitudinal

axis of the horizontal root. This plan of construction was rendered visible to the naked eye by patterns on the bark, by growth-rings or metatracheal parenchyma, and by injecting the vessels with a solution of eosin.

With increasing growth in height and thickness, the anatomy of the wood and the types of symmetry displayed become too complex to be summarized, and the pneumatophore becomes a kind of semi-symmetrical burr. But its developmental trend may be indicated by the statement that, when the growth of the pneumatophore is most regular, the wood begins as a structure that is isobilateral with its longitudinal axis of symmetry horizontal, but it ends in the outer proximal parts of the pneumatophore by being nearly or quite radially symmetrical with the longitudinal axis of symmetry erect, while in its distal parts it retains its bilateral symmetry.

The medullary rays in regions where the course of the vessels and fibres is straight are thinner and taller than where the course of the latter is curved. The medullary rays in planes other than the median vertical plane (of the main root), as they pass outwards, execute remarkable twists in planes tangential to the pneumatophore.

As regards the physiological anatomy of the pneumatophores, all possessed lenticels (the secondary cortex was too disorganized in our specimens to permit of proper examination). The wood-vessels are relatively scanty : they were partly blocked by callus-like plugs in *Amoora* and less localized excreta in the two other species ; nevertheless, they were easily injected with aqueous solutions of eosin. Thick-walled fibres are not present in great amount. Thin-walled fibres and wood-parenchyma, or the latter alone, together with parenchyma of the medullary rays, formed the main mass of the wood. Although in some parts the wood-parenchyma and some ray-cells contained abundant starch, and thus at times represent no inconsiderable food-reservoirs, these tissues as well as the fibres largely had no solid contents. It is thus possible that all the cells, except the youngest, and the vessels may act as air-reservoirs. Only in *Heritiera* were the intercellular spaces in the wood somewhat larger than usual.

Fungal hyphae occurred in the wood and secondary cortex of all three species, but may have represented a post-mortem invasion.

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EXPLANATION OF PLATES II AND III.

Illustrating Messrs. Groom and Wilson's paper on *Amoora, Carapa*, and *Heritiera*.

PLATE II.

Figs. 1–5. *Amoora cucullata.*

Fig. 1. Portion of the horizontal root and an intact pneumatophore showing at the base external traces of the arch-like structure. Reduced.

Fig. 2. Section of wood of part of a pneumatophore taken in the median vertical radial plane (of the horizontal root). For explanation see Text-fig. 3. Magnification, 5 diam.

Fig. 3. Section of wood of the top of a pneumatophore taken in the middle in a plane transverse to the horizontal root. For explanation see Text-fig. 4. Magnification, 5 diam.

Fig. 4. Tangential section of wood of portion of a pneumatophore taken in a plane parallel to the vertical radial plane of the main root. For explanation see Text-fig. 7. Travelling from left to right the medullary rays in tangential section are successively oblique, prostrate, and erect. Magnification, 25 diam.

Fig. 5. Cross-section of the more central part of the wood of a pneumatophore, showing the inmost growth-ring and part of an outer ring. For explanation see Text-fig. 6. Magnification, 25 diam.

PLATE III.

Figs. 6–9. Pneumatophores of *Carapa moluccensis* var. *gangetica.*

Fig. 6. Cross-section of a portion of the outer parts of the wood showing irregularity of growth in thickness : all the wood is cut in cross-section. Magnification, 7·3 diam.

Fig. 7. A tangential section in a plane transverse to the horizontal root, showing the wood in cross-section on the left half, and in radial longitudinal section on the extreme right hand. Magnification, 25 diam.

Fig. 8. A tangential section in a plane parallel to the vertical radial longitudinal plane of the horizontal root, showing arch-structure, curved course of the fibres and vessels, and the thick medullary rays. Magnification, 25 diam.

Fig. 9. Wood with straight course of the vessels, tangentially cut, showing the rays thinner than in Fig. 8, and near the right-hand edge a long so-called 'resin' gland. Magnification, 25 diam.

Figs. 10–12. Pneumatophores of *Heritiera minor.*

Fig. 10. Tangential section of the wood in a plane parallel to the median vertical radial plane of the horizontal root, and showing the arch-structure so irregular as to become burr-like. Magnification, 25 diam.

Fig. 11. Cross-section of the outer part of a pneumatophore in a region that had five growth-rings ; the section shows the wood transversely cut, and three outer growth-rings together with part of an inner one. Magnification, 25 diam.

Fig. 12. Cross-section of part of the pneumatophore illustrated in Fig. 11, but taken in the inmost growth-ring ; the wood is everywhere cut in a longitudinal tangential direction. Magnification, 25 diam.

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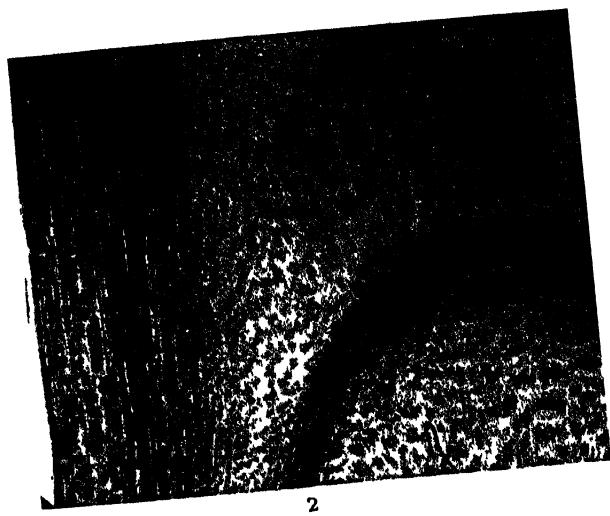
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GROOM AND WILSON—PNEUMATOPHORES.

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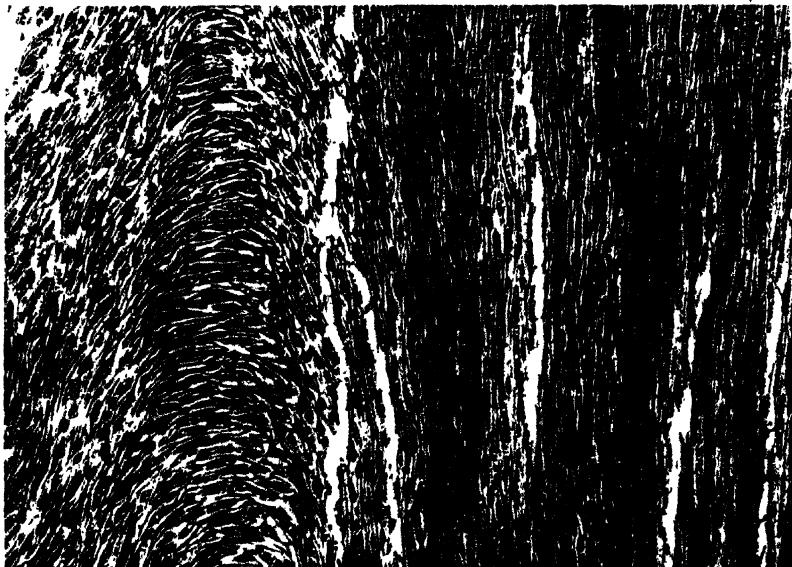
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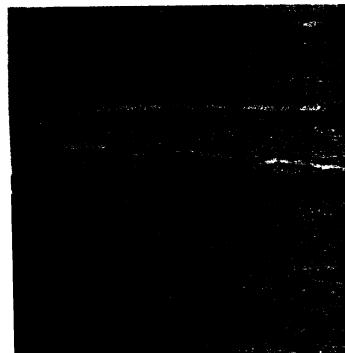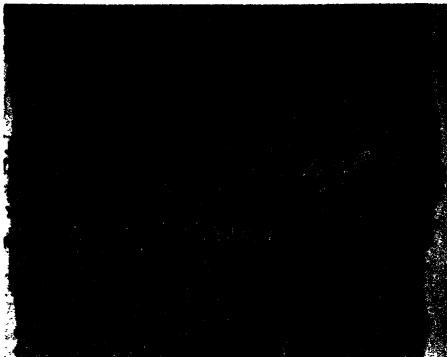
Observations on the Anatomy of Teratological Seedlings.

V. On the Anatomy of some Atypical Seedlings of Sinapis alba and Brassica oleracea.

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With sixty-six Figures in the Text.

IN previous papers of this series the anatomy of polycotylous seedlings of *Cheiranthus Cheiri* (6) and *Centranthus ruber* (1) has been described, and the theory has been put forward that polycotyly may arise either by cotyledonary fission or by dichotomy of the growing-point of the cotyledon. The vascular strands of two cotyledons originating by the first method are associated with a single root pole, but in cotyledons originating by the second method there is a tendency for each vascular strand to be associated with a separate root pole. If diarchy obtains in the normal seedling, therefore, a tricotylous seedling of this second type will develop a triarch stele, this being either a transient stage at the upper end of the hypocotyl, or persistent in the hypocotyl and the whole or major portion of the root.

In *Centranthus ruber*, however, polycotylous seedlings have been examined which in their anatomical structure differ from those of either of the above-mentioned categories, since the vascular strand of one cotyledon does not contribute towards the formation of the root stele. The origin of cotyledons of this type has not been satisfactorily explained.

The present study was undertaken with the idea of supplementing the evidence already obtained concerning the origin of polycotyly. In view of the very characteristic form of the normal cotyledon in the Cruciferous seedlings it seemed possible also that some connexion might be traced between the form of the cotyledon of the polycotylous seedling and its method of origin, although the great plasticity exhibited by this organ rendered this somewhat improbable.

During the course of the investigation other atypical seedlings were found, and the results of the examination of these are also recorded, although for the most part they have no direct bearing on the main problem.

Seedlings of two species, *Sinapis alba* and *Brassica oleracea*, have been examined, but the structure of these seedlings is so similar that it has not been thought necessary to describe them separately.

The Normal Seedling.

The cotyledons of the normal seedling are petiolate, the two petioles being usually markedly unequal in length. The cotyledon lamina is short
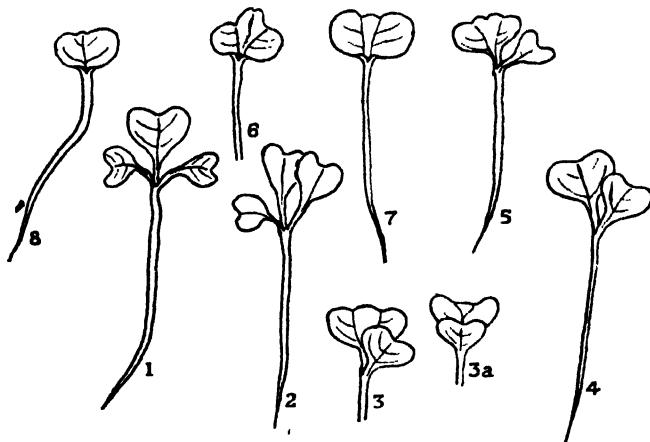
FIGS. 1–8. Figs. 1 and 2. Tricotylous seedlings showing variations in length of cotyledon petioles. Figs. 3, 3 *a*, and 5. Hemitricotylous seedlings showing different types of lobing of the abnormal cotyledon. Fig. 4. Normal seedling. Figs. 6–8. Syncotyls A, B, and C.

and broad, with two well-marked lobes (Fig. 4), and possesses a midrib bundle which terminates in the central depression between the lobes.

The anatomical structure of *Brassica oleracea* has been described by Chauveaud (2), so that no detailed description of the normal seedlings need be given here. The cotyledon petiole possesses at the base a median bundle consisting of two groups of metaxylem and phloem, one on either side of the central protoxylem group, and two small lateral bundles. The lateral strands may, in both species, fuse with the median bundle either slightly above or at the cotyledonary node, or they may enter the hypocotyl as independent strands. In the hypocotyl the two principal bundles lying in the cotyledonary plane undergo ' rotation ' and constitute the two poles of the diarch root stele, in the formation of which the lateral strands play no part.

Tricotyls.

In the tricotylous seedlings each of the three cotyledons agrees exactly in form with those of the normal seedling. The inequality in the length of the cotyledon petioles is again well marked, but whilst in some specimens two cotyledons are longer than the third (Fig. 2), in others the opposite condition obtains (Fig. 1). Frequently all three cotyledons are of equal size, but occasionally two are somewhat smaller than the third. Of the thirty-six seedlings examined all but two are of the same general type, and for these a brief general description will suffice.

The petiole of each cotyledon contains a median and two lateral strands, the latter either fusing with the median strand or persisting into the hypocotyl as independent bundles, in which case they either ultimately fuse with the adjacent median bundle or simply die out. The behaviour of the laterals is variable in a single seedling, and even the fellow laterals of one cotyledon frequently vary somewhat in their time of fusion. In the hypocotyl a triarch structure obtains, and this, in many seedlings, persists throughout the root, but in a few specimens reduction to diarchy by fusion of poles occurs at a low level in the hypocotyl or in the root.

The two remaining tricotyls are quite different in structure from those just described, although indistinguishable in external form. Both are seedlings of *Sinapis alba*, and will be designated tricotyls B and C.

Tricotyl B. The three cotyledon petioles have the normal structure, each possessing a median and two lateral strands (Fig. 9). Two of the cotyledons undergo a somewhat precocious lateral fusion (Fig. 10), and in these cotyledons fusion of the lateral strand with the midribs occurs at an early stage on the symphysis side, while the outer laterals are more persistent, one extending into the hypocotyl before fusion. In the hypocotyl the vascular strand connected with the cotyledon, which remains separate until the general fusion at the cotyledonary node, behaves in the normal manner, the phloem group dividing into two portions, while the protoxylem assumes the exarch position. The two bundles belonging to the cotyledons which fuse prematurely are in close proximity to one another at the other end of the somewhat elliptical stele (Fig. 12), and behave in an unusual manner. In one bundle there is an unequal bifurcation of the phloem accompanied by the usual assumption of the exarch position by the protoxylem. The metaxylem groups of this bundle are also markedly unequal in size. The second bundle remains endarch in structure throughout its course, and is situated on that side of the adjacent bundle which shows reduced metaxylem and phloem (Fig. 12). The endarch bundle gradually approaches the small adjoining metaxylem and phloem group, and ultimately the metaxylem and phloem elements fuse with it, whilst the protoxylem

disappears (Fig. 13). A typical diarch stele is therefore present in the lower region of the hypocotyl and in the root. Two leaves are borne at the first epicotyledonary node in this seedling.

Tricotyl C. Immediately above the cotyledonary node the three petioles show the normal structure, each possessing a median strand and two laterals (Fig. 15). In two of the cotyledons the lateral strands fuse with the median one just prior to the entry into the hypocotyl, but in the third cotyledon one lateral remains independent for a short distance in the hypocotyl.

Of the three vascular strands present in the upper portion of the
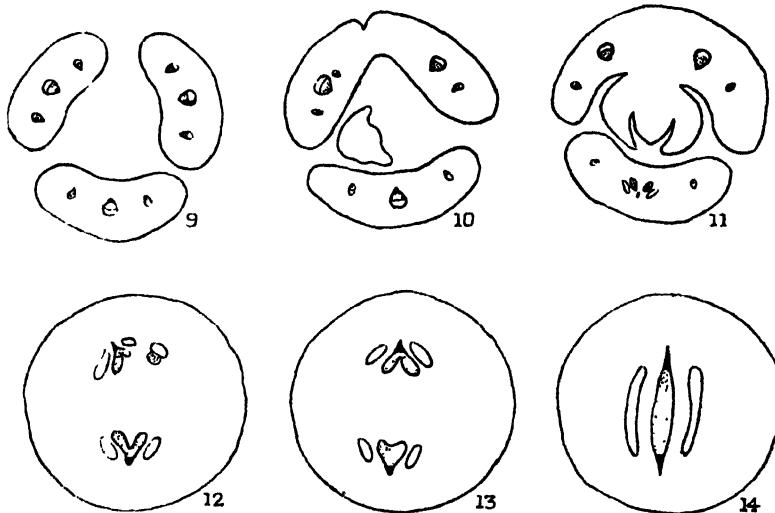
FIGS. 9–14. Diagrams showing transition phenomena in tricotyl B.

hypocotyl two behave in the normal manner and give rise to two root poles, but the third retains its collateral endarch structure throughout its course. Immediately below the cotyledonary node all three bundles are of equal size and are symmetrically placed in the stele (Fig. 17). At a lower level in the hypocotyl the two normal bundles form a diarch xylem plate, whilst the abnormal xylem group loses its protoxylem and moves inward until it comes in contact with the xylem plate (Fig. 18). It decreases gradually in bulk and finally disappears (Fig. 19). There is, therefore, in this strand neither fusion with either of the normal strands, such as occurred in seedling B, nor any attempt at root-pole formation. Three leaves are present at the first epicotyledonary node in this seedling (Fig. 15).

One other tricotylous specimen exhibits some features of interest and must be described in some detail. This seedling (*B. oleracea*) is an amphitrisyncotyl, but each cotyledon possesses at the base the typical number of

strands, a median one and two laterals. These strands all enter the hypocotyl separately and a triarch stele is produced (Figs. 20–22), the three xylem groups meeting to form a solid xylem core. At a lower level, by the
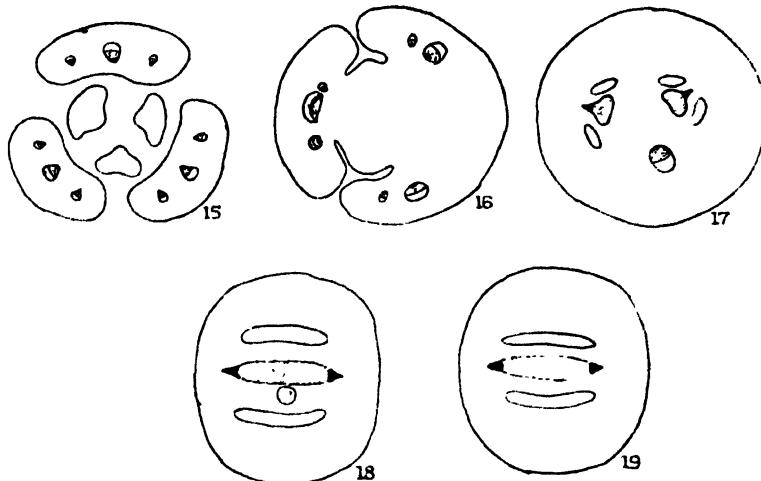
FIGS. 15–19. Diagrams showing transition phenomena in tricotyl C.
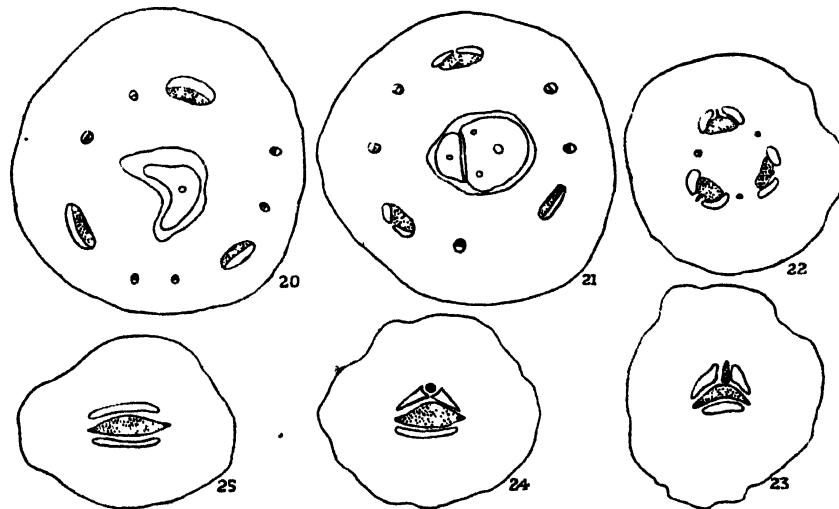
FIGS. 20–25. Amphitrisyncotyl. Figs. 23–25 show stages in elimination of one xylem group.

disappearance of a few metaxylem elements, one xylem arm becomes separated from the others (Fig. 23), these, as the result of a slight displacement, forming a normal diarch bar. The phloem groups which lie on either side of the detached xylem become extended laterally until the xylem mass

is almost isolated (Fig. 24). At this stage cambial activity is evident, and at a slightly lower level a continuous layer of cambium extends on either side of the diarch xylem bar, so that cambium and phloem tissue separate the isolated xylem mass from the remainder of the stele (Fig. 26). This condition prevails until the disappearance of the isolated xylem, which is not long delayed.

Hemitricotyls.

In external form the hemitricotyls are variable. In some seedlings the abnormal cotyledon has a median cleft of variable depth, each half lamina being lobed in the manner characteristic of the normal cotyledon (Fig. 5). Each half lamina has a midrib with normal outer laterals, but with the inner laterals in many cases less strongly developed. The median cleft in the cotyledon may involve only a small portion of the lamina or it may extend to the petiole. In other seedlings there is a small central lobe in addition to the two lateral ones, this being accompanied by a very obvious doubling of the midribs (Fig. 3), while in others, again, there may be only a very small central lobe or even merely a flattening of the median depression, and a less marked bifurcation of the midribs (Fig. 3 a).

In seedlings with a median cleft in the cotyledon the hypocotyl and root almost invariably possess a triarch structure, but in one seedling in which the median cleft extends to the cotyledon petiole the two strands belonging to the bifid cotyledon rotate so as to form one pole of the diarch root stele. Seedlings with a median lobe are sometimes triarch and sometimes diarch, but the latter condition is the more typical.

One hemitricotylous seedling merits more detailed description, since in general structure it resembles the tricotyl described as tricotyl C. This hemitricotyl is one possessing a median lobe (Fig. 3). Just above the cotyledonary node the abnormal cotyledon possesses two large bundles of practically equal size and two laterals situated one on the outer side of each large bundle. In one of the principal strands the phloem undergoes bifurcation and the protoxylem becomes exarch* in position, whilst the other retains its collateral structure. After entry into the hypocotyl the one bundle proceeds to form one pole of the diarch root, while the other remains collateral. At first the latter is at one end of the stele, almost in contact with the adjacent bundle, but it ultimately lies approximately midway between the two xylem poles. The abnormal xylem group diminishes in size, but a few metaxylem elements persist even in the root.

In *Sinapsis alba* one apparently tetracotylous specimen was found, but this, on examination, proved to be a 'twinned' seedling resembling in general structure the *Centranthus ruber* specimen previously described (1). Two sets of plumular leaves are present, and each of the four cotyledons is

quite normal in structure (Fig. 27). In the upper portion of the hypocotyl two steles are organized, each cotyledonary bundle consisting of an exarch xylem mass flanked by two phloem groups. In each stele the epicotyledonary strand is especially conspicuous on the inner side (Figs. 29 and 30). The phloem groups on the outer side of each stele fuse to form one group
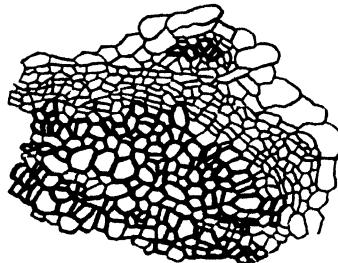
FIG. 26. Amphisyncotyl hypocotyl showing isolated xylem group separated from the diarch plate by cambium and phloem tissue.
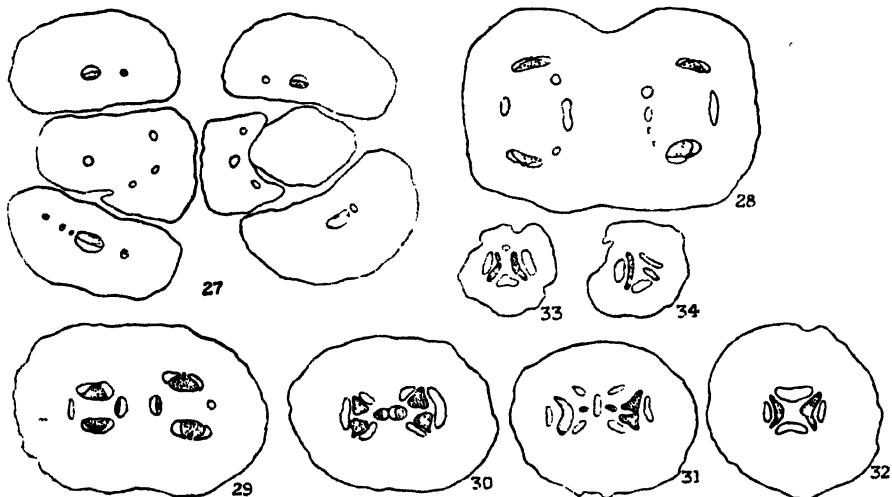
FIGS. 27-34. Transition phenomena in a 'twinned seedling'.

in the intercotyledonary plane, but the inner phloems remain more or less in their original positions, and the adjacent groups, one from each stele, undergo fusion, the epicotyledonary phloem also fusing with the cotyledonary phloems (Figs. 31 and 32). The two diarch xylem plates are somewhat curved so that the convex sides are adjacent. In the root one phloem group undergoes reduction and finally disappears, this being accompanied by the disappearance of one xylem pole, so that the root tip contains a single triarch stele (Fig. 34).

Syncotyls.

Three syncotylous specimens of *Sinapis alba* have been examined, these forming a series showing progressively closer fusion of the cotyledon laminae (Figs. 6–8). The study of these seedlings is of interest, since hitherto the phenomenon of syncotyly has been mainly studied in seedlings with tetrarch or triarch structure (3, 5, 8).

Syncotyl A. The form of the cotyledon in this seedling suggests very
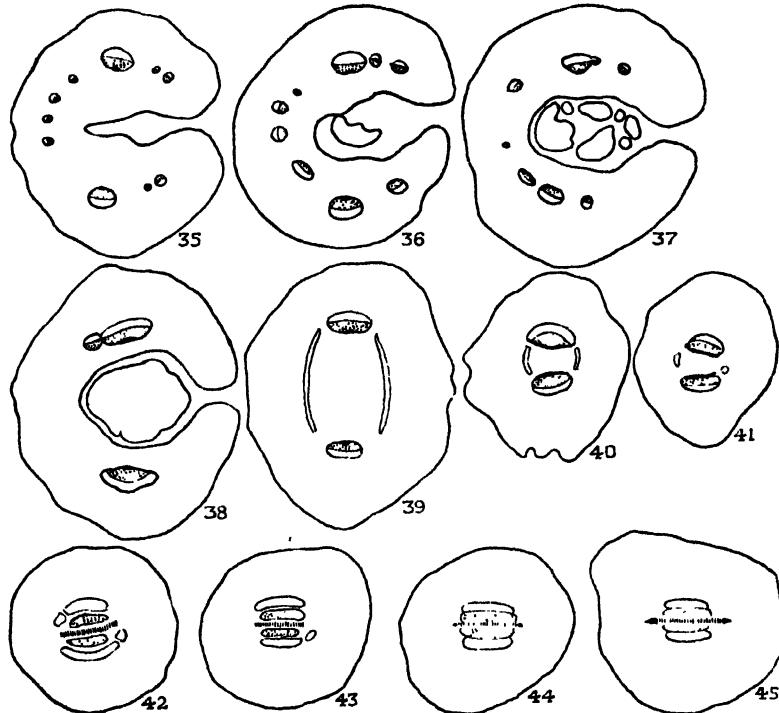
FIGS 35–45. Transition in syncotyl A, showing abnormal behaviour of cotyledonary strands, and formation of diarch plate in the intercotyledonary plane.

definitely its origin by the lateral union of two cotyledons (Fig. 7). Two midrib strands are present in the lamina, and these are widely separated even in the petiole (Fig. 38), so that on entrance into the hypocotyl they occupy opposite ends of the elliptical stele (Fig. 39). Lateral strands are present at the base of the petiole on either side of each midrib, but these fuse with their respective midribs before the cotyledonary node is reached (Figs. 37 and 38). The epicotyl is well developed, the leaves of the first two nodes being visible. There is a marked difference in size of the two leaves at the first node, the larger leaf appearing on the symphysis side,

whereas in other syncotyls in which the form of the epicotyl has been studied (5, 8) the reduced leaf appeared on the symphysis side.

At the summit of the hypocotyl there are present the two large strands derived from the cotyledon, with two elongated bars of epicotyledonary vascular tissue extending between them. The strands connected with the epicotyl are not well differentiated, no lignified xylem elements being evident. At lower levels in the hypocotyl the epicotyledonary strands, consisting of phloem only, become reduced in size and finally fuse with the phloem of the cotyledonary bundles (Figs. 40–43). Meanwhile the cotyledonary strands, instead of 'rotating' in the normal manner, have retained their collateral structure and have merely undergone a lateral extension, a line of crushed elements being visible on the inner margin of each bundle (Fig. 41). The bundles move gradually inward, while a central file of elements occupies the median line *in the intercotyledonary plane* (Fig. 42). These elements are apparently at this level partly of recent origin and partly derived from the inner margin of the cotyledonary bundles.

At a lower level a definite diarch plate is organized in the intercotyledonary plane, the protoxylem elements arising *de novo*, whilst the two cotyledonary bundles lie on either side, each consisting of a few xylem elements and a group of phloem. Unfortunately the seedling was damaged when collected, so that the structure of the root cannot be described.

Syncotyl B. In this seedling the double nature of the cotyledon is still evident, but the fusion is a fairly close one (Fig. 6) The two midrib strands are distinct in the lamina and upper portion of the petiole, but subsequently they undergo fusion so that just prior to the passage into the hypocotyl five strands are present. namely, the strand resulting from midrib fusion, with a lateral and marginal strand on either side (Fig. 48). The marginal and lateral strands quickly fuse, so that in the hypocotyl just below the cotyledonary node one large and two small bundles are present (Fig. 50).

The phloem of the large bundle divides into three portions, and two groups of exarch protoxylem appear, so that a curved, laterally placed diarch xylem plate is formed with a large phloem group between the two protoxylem groups and a smaller phloem on either side (Fig. 51). Meanwhile the two small strands gradually approximate and finally fuse to form a collateral bundle lying at one side of the asymmetric diarch plate (Figs. 51 and 52). The two smaller phloem groups associated with the diarch plate move across and ultimately fuse with the phloem of the collateral bundle, while the xylem of this bundle moves inward until it comes in contact with the xylem bar (Fig. 54). At this stage the diarch plate has straightened somewhat, but shows a protuberance at the side where fusion with the other bundle has taken place. Ultimately a normal diarch stelar structure is attained (Fig. 55).

Syncotyl C. The seedling has apparently a single cotyledon, but the terminal lobing is not quite normal in form (Fig. 8). The petiole possesses a median strand with several lateral strands on either side. These fuse into one composite bundle towards the base of the petiole, so that one median strand and two fairly large laterals enter the hypocotyl (Fig. 61).
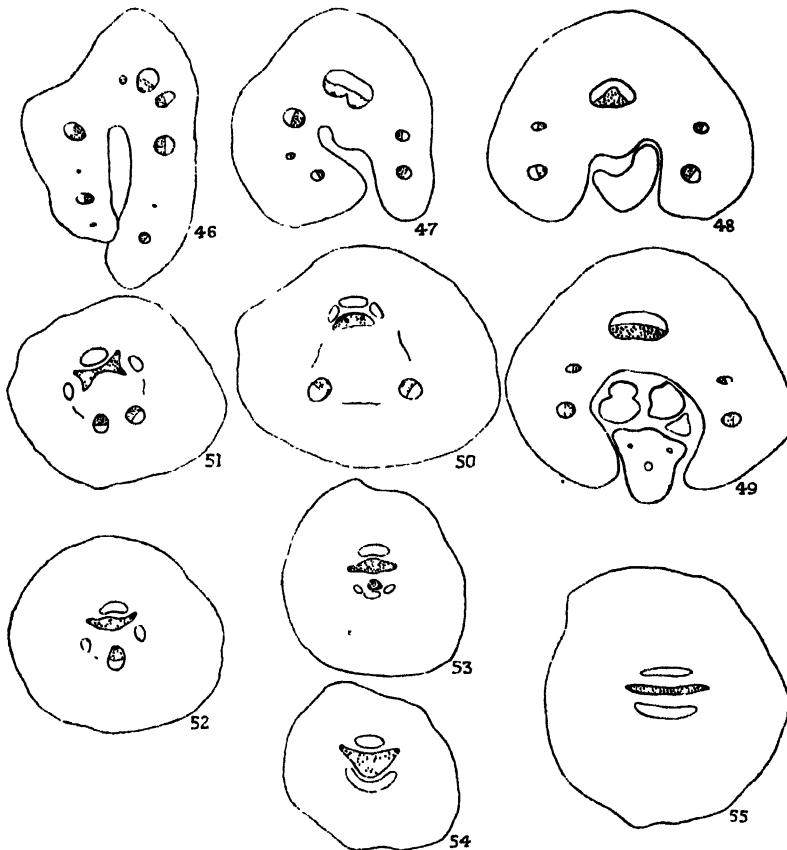
FIGS. 46-55. Transition in syncotyl B.

In the median strand the protoxylem has assumed the exarch position even above the cotyledonary node, and a normal pole is organized fairly quickly. The two lateral strands move towards one another and fuse, combining with a small epicotyledonary strand which lies between them (Fig. 62). The phloem thus forms an arc round the composite bundle, while the proto-xylem retains its endarch position until its disappearance (Fig. 63).

This condition prevails throughout the hypocotyl and the upper portion of the root, lateral roots being developed in connexion with the one normal pole (Fig. 64). At a lower level in the root the phloem undergoes division

opposite the metaxylem group, and a protoxylem group appears, so that a diarch stele is produced (Fig. 65). A lateral protuberance then appears in the metaxylem near the first-formed pole, followed by the development of a third protoxylem, so that at the root tip the stele is triarch, although no phloem group has yet developed between the latest formed xylem group and the adjacent one (Fig. 66).

In both seedling B and seedling C the epicotyl shows modification as
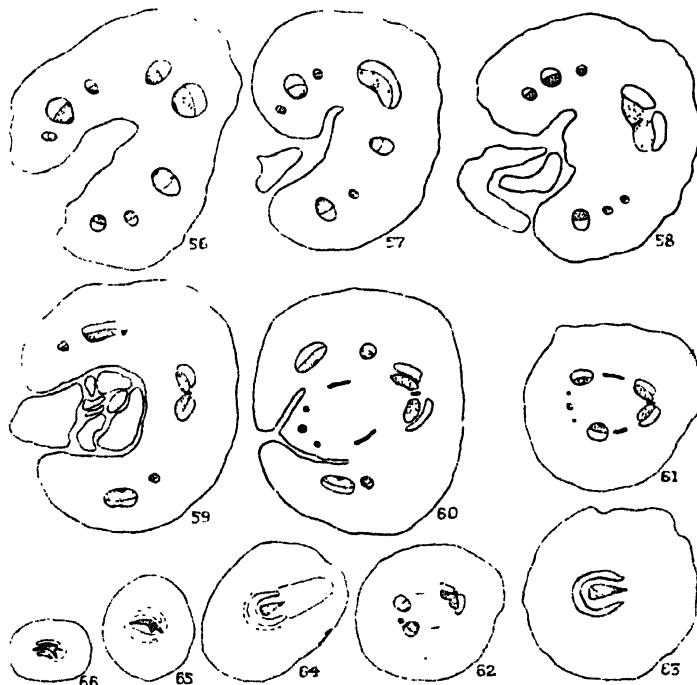
FIGS. 56–66. Transition in syncotyl C.

the result of the syncotylous condition, since at the first epicotyledonary node only one leaf is developed, this being on the side remote from the symphysis (Figs. 49 and 59).

DISCUSSION.

The polycotylous seedlings of *Sinapis alba* and *Brassica oleracea* examined in the present investigation show the same two modes of transition as do the seedlings of other species previously described, since in some specimens two cotyledonary strands together constitute a root pole, whilst in others each strand links up with a separate pole. A small percentage of the seedlings show the peculiar mode of transition in which the strand of

one cotyledon takes no part in root-pole formation. This mode of transition has apparently been observed hitherto only in *Centranthus ruber* among Angiosperm seedlings (1), although a somewhat similar phenomenon has been recorded in Gymnosperms (4). The structure of the tricotylous seedling of *Sinapis* described above under the title tricotyl B would seem to suggest the possibility, already discussed in connexion with *Centranthus ruber*, that the abnormal cotyledon of such seedlings might be produced by the unequal fission of the original cotyledon. The midrib strand would therefore be equivalent to the lateral strand of a normal cotyledon, which, as has been stated, may enter the hypocotyl even in the dicotylous seedling.

Tricotyl C. with a symmetrical arrangement of the strands in the hypocotyl and the absence of any association of the aberrant strand with the adjacent strand, does not, however, lend any support to this theory. A further objection is to be found in the form of the cotyledon in the hemitricotylous seedling, in which the same behaviour of one strand is found. If the suggested mode of origin is the actual one, it might be expected that a hemitricotyl would give some more clearly marked indication of the relationship existing between the two strands. This, however, is not so, as there is no indication of unequal fission, and no difference in the course of the two strands.

Some further light seems to be thrown on the problem by the structure of the seedling described as syncotyl A. This seedling shows a most extraordinary structure which seems to be without any well-accredited parallel. Judging by the form of the lamina the seedling is one showing an early stage of syncotyly, with the midribs widely separated and entering the hypocotyl approximately in their normal position. The idea that the two strands in question are midrib strands occupying the normal cotyledonary plane is strengthened by the position of the epicotyledonary leaves. The failure of the cotyledonary strands to play any important role in transition, together with the formation of the diarch plate in the intercotyledonary plane, are phenomena which have not been observed elsewhere, with one doubtful exception. Kattein (9) described the seedling of *Lupinus luteus* as having the root plate in the intercotyledonary plane, the polar protoxylems passing up into the epicotyl, whilst the lateral collateral bundles enter the cotyledons. This has, however, been disproved for the normal seedling of *Lupinus luteus* at least (7).

If the cotyledonary strands of syncotyl A are midrib strands, and of this there seems to be little doubt, the seedling displays the extraordinary phenomenon of the cotyledon midribs taking no part in root-pole formation. In view of this there seems to be no valid objection to the interpretation of the abnormal strands of tricotyls B and C and the hemitricotyl as strands equivalent to those of the other cotyledons, but which for some unknown reason are failing to participate in root-stele organization.

It must be admitted that the existence of such exceptional forms casts some doubt on the value of the behaviour of the cotyledonary strands in transition as a reliable criterion of cotyledon origin. The possibility remains, however, that further investigation may result in the discovery of strands intermediate in behaviour between the abnormal ones under discussion and the more normal type.

The absence of any close correlation between the form of lobing of the abnormal cotyledon in polycotyls and the behaviour of the cotyledonary strands in transition is very clearly demonstrated by the tricotylous and hemitricotylous seedlings described in the present paper.

There remain to be discussed the syncotylous seedlings A and B. The study of syncotyly in this species is of interest in view of the diarch symmetry of the normal seedling. Previous work on syncotyly has dealt chiefly with seedlings possessing triarch and tetrarch symmetry in which lateral strands are normally involved in root-pole formation (3, 5, 8). Dr. E. N. Thomas (10), however, has recorded syncotyly in some species of the Ranunculaceae in which diarch symmetry obtains in the normal seedling. The method of organization of the root stele is not uniform in these seedlings. In the syncotylous specimen of *Anemone pulsatilla* the two vascular bundles present in the base of the cotyledon fuse and together constitute one pole of the diarch root, whilst the strand from the first plumular leaf is connected with the second root pole ; in *Clematis Davidiana*, on the other hand, the root stele is apparently connected solely with the cotyledonary strands. The account given of this seedling is, however, not sufficiently detailed to make comparison with the *Sinapis* syncotyls possible.

In seedlings with tetrarch symmetry as described by Compton (3), Holden (5), and Holden and Daniels (8), syncotyly results in the suppression of the lateral strands on the symphysis side, and in the case of some *Impatiens* seedlings in fusion of the midrib strands so that they jointly form one root pole. There is also in many *Impatiens* syncotyls an increase in the number of root poles as the result of the increased importance of the lateral and marginal cotyledonary strands, the marginals fusing to form a normal root pole, whilst the lateral remains independent and gives rise to an additional root pole.

Although the syncotylous *Sinapis* seedlings which have been examined are too few in number to permit of general conclusions being drawn, it seems evident that the same tendencies are operating here as in the syncotyls previously studied, namely, a tendency for close syncotyly to lead to midrib fusion and to an increased importance of the lateral strands.

In the normal *Sinapis* seedling the lateral strands may persist below the cotyledonary node, but they die out or fuse with the adjacent bundle at a very high level in the hypocotyl and play no part in the transition

phenomena. In syncotyl B, on the other hand, the two laterals fuse in the hypocotyl, forming a fairly large strand which remains independent for a short distance, but finally fuses with the diarch plate formed by the two midrib bundles. A further advance is shown by syncotyl C, in which the fused lateral strands, after persisting throughout the hypocotyl as a group of metaxylem elements, become associated in the root with protoxylem elements and constitute a root pole.

The tendency towards midrib fusion is equally well illustrated by these seedlings. Thus, in seedling B, the midrib bundles fuse in the cotyledon petiole, but in transition the double origin of the composite bundle is clearly evident, and ultimately a diarch plate is formed in the normal cotyledonary plane. In seedling C there are no well-marked indications of the double nature of the cotyledon, the midrib being apparently single, but taking into consideration the slightly irregular lobing of the lamina, the tendency towards midrib fusion shown by syncotyl B and by syncotyls of other species, and the position of the first epicotyledonary leaf in the seedling, it seems most probable that the specimen is one of syncotylous type. On this view there has been complete fusion of the midribs, which form a single pole persisting throughout the hypocotyl and the major portion of the root. The development of a third pole in the extreme root tip may be an attempt to revert to the original symmetry.

SUMMARY.

1. The anatomy of polycotylous and syncotylous seedlings of *Sinapis alba* and polycotylous seedlings of *Brassica oleracea* is described.

2. The significance of the failure of one cotyledonary strand of a polycotylous seedling to participate in transition is discussed, together with the value of the behaviour of cotyledonary strands in general as a criterion of cotyledon origin.

3. The influence of the syncotylous condition on the anatomy of the seedling is compared with that noted in syncotylous specimens of other species.

4. A 'twinned' seedling of *Sinapis alba* is also recorded.

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Life-history and Cytology of Rhytisma acerinum (Pers.) Fries.

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With Plate IV and twenty-three Figures in the Text.

INTRODUCTION.

THE early investigators who interested themselves in the 'wrinkled scabs' ('Runzelschorfe') on the leaves of various species of *Acer*, due to the action of *Rhytisma acerinum*, confined their work almost entirely to the discovery of biologic forms of this parasite. Julius Müller (26) in 1893 attempted to establish on morphological grounds that *R. acerinum* embraced several species, and he discovered gradual variations, which he classified under four types according to the relative diameter of the stromata. The smaller-sized stromata, he stated, were found only on the taller Maple trees, whereas the larger stromata occurred on the small bushy trees. These differences in the size of the stromata he attributed to the relative number of spores carried from the ground. He also gave some interesting anatomical details of the structure of the stroma, stating that in sections cut parallel to the surface of the leaf a net-like appearance (presumably the outline of the infected epidermal cells) was seen, and that the stroma was covered in by the upper half of the ruptured epidermal cells. Earlier references are found under the names of Fuckel (17) and Rostrup (31), both of whom had also considered it probable that biological forms of this fungus existed. Karl Müller (27) in 1912 carried out investigations on the problem of biologic forms on strictly scientific lines by means of actual inoculations with ascospores. He experimented on the Norway Maple, Field Maple, and Sycamore Maple. Having carried out his inoculation experiments on these trees in the open, and also on small pot trees under glass, he found that the spores from Norway Maple successfully infected the Field Maple; but when conveyed to the Sycamore Maple

infection was very slight, if any at all. Spores conveyed from the Sycamore Maple failed to infect either Field or Norway Maples; and again, spores from the Field Maple attacked the Field Maple itself very strongly, the Norway Maple weakly, but failed on the Sycamore Maple. For the purposes of inoculation, Müller first sprayed the plants and applied the ascospores in water to both the upper and under surfaces of different leaves by means of a brush. He found that almost without exception the leaves were infected only when inoculated on the under side, and though he made no sections of such leaves he conjectured that the germ-tubes entered by means of the stomata. Müller was able to procure infection at the upper surface, but only when the ripe stromata were pressed down vigorously on the epidermis to such an extent as to rupture the thick cuticle. He also made careful observations to determine the length of time taken from inoculation to the appearance of the familiar yellow patches on the leaves, and found that it varied considerably in different years according to seasonal variations of moisture and warmth. In the open air the time taken averaged about eight weeks, but in a glass-house it only took from four to six weeks. He also gave some interesting details of the weather conditions necessary for spore-discharge from the stromata, stating that the expulsion of spores was greatly favoured by alternation of damp and dry weather. He finally described, with figures, the ascospore as being furnished with a jelly-like exine—an observation which had previously been described by Klebahn (24) but contradicted by J. Müller. Klebahn had also described the phenomenon of spore-discharge, and said that the gelatinous sheath functioned as a means whereby the spores could be carried by wind to the leaves to which they adhered by means of the gelatinous covering. K. Müller (27, 28) thus gives us a fairly exhaustive account of the biology of *Rhytisma acerinum*, together with descriptions and figures of stromata, spermatia, asci, and ascospores. The details of development and cytology of this parasite have, however, not been described, and at the suggestion of Professor Lloyd Williams the writer has attempted an investigation of the structure of this fungus, together with a study of the cytological features presented in the ascus.

Brief Outline of the Life-History.

Rhytisma acerinum occurs on the leaves of Sycamore (*Acer pseudo-platanus*), forming on the upper surface black wrinkled stromata, roughly circular in shape, each with an average diameter of about 15 mm. The mycelium of uninucleate cells fills the leaf-cells immediately below and in the vicinity of the stroma, forming in the cells of the upper epidermis a dense mass of hyphae. The two fructifications—the pycnidia and the apothecia—develop entirely within these ruptured epidermal cells. The

pycnidia, which are the first to be formed, appear towards the centre of the stroma and give rise to minute conidia. The apothecia are developed later, usually at the margin of the stroma, giving the latter a much wrinkled appearance. After the pycnidia have become inactive, but often before their complete disappearance, there are found in the apothecia the rudiments of several archicarps. An archicarp when finally formed is of the nature of a scolecite, in which one cell only gives rise to ascogenous hyphae, the ramifications of which are exceedingly complex. At first the asci arise in groups, but later they are seen in close formation amongst dense ranks of straight paraphyses. The young ascus shows a pair of associated nuclei, the fusion of which is delayed. At all three divisions in the ascus five chromosomes are found (Maire (25 *a*) discovered four chromosomes). The fully formed ascospore is filiform and unicellular, showing at its centre a distribution of chromatin extending for about a fourth of its entire length. It is furnished with a massive sheath, and at germination soon becomes septate before giving rise to a germ-tube (Text-fig. 2, *a*).

Spore-Discharge and Infection Experiments.

Towards the end of March stromata were brought into the laboratory and placed in Petri dishes containing a small quantity of damp soil. After a few days some of the wrinkled apothecia, when examined with a lens, showed signs of rupture of the roof along a median line, and the following day there emerged through these fissures a yellowish gelatinous substance and, in addition, numerous other apothecia showed signs of approaching dehiscence. Some hours later those with the gelatinous exudate had opened considerably wider. A stroma in this condition was now taken, and, in a darkened room, held in a pair of forceps in a beam of light from a point-light lamp. For some seconds one could observe the gradual diminution of the glistening appearance presented by the yellow exudate, accompanied by contraction of the exudate and its withdrawal into the fissure. At the same time the fissure itself widened very gradually, and finally a small cloud of ascospores was shot out. This was repeated at intervals, but other apothecia followed intermittently in discharging their spores, and owing to this difficulty one could not with certainty confine attention to one particular apothecium, in order to determine whether there was any strict periodicity as to the intervals between successive discharges. Even after the expulsion of a definite cloud of spores there occurred in the same apothecium feeble puffs at frequent intervals. After repeated observations it was concluded that some twenty minutes elapsed before the same apothecium discharged another cloud. It must, however, be remembered that this long exposure to the warm, dry air of the laboratory would cause the apothecium to become too dry, for when the stroma was

returned to the Petri dish, immediately after a maximum discharge, and left for five minutes, the same apothecium on exposure puffed again most vigorously. A puffing apothecium will show asci and ascospores at all stages of development. Thus one can see in microtome sections bundles of ascospores escaping from the hymenium, and below them young developing asci, together with others showing the three sets of spindles.
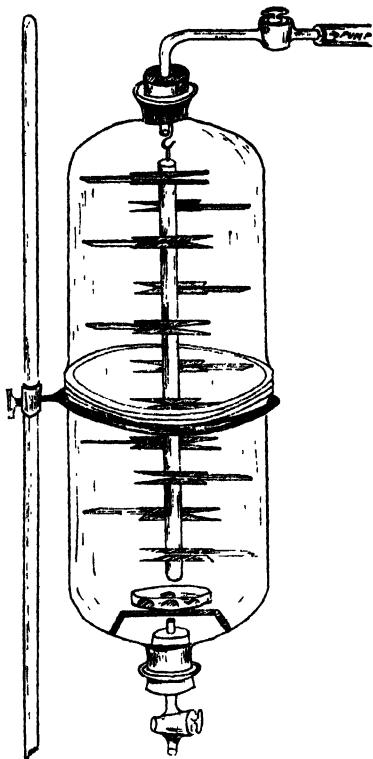
It is thus seen that an apothecium remains functional for a comparatively long period. In about a fortnight after rupture an apothecium is fully open, and at this time it is of a pale buff colour, still showing a glistening surface. The disappearance of the latter indicates that the apothecium is no longer functional.

Though Müller (28) has correctly stated that the ripe spores are only shot to a height of about 1 mm. from the surface of the apothecium and thereafter conveyed upwards by air-currents, we still find reference in the text-books to the fact that the spores are ejected to a considerable distance. In order to test this point the apparatus figured (Text-fig. 1) was set up. This consists of a number of slides coated on both sides with a thin solution of gelatin, and fixed in a horizontal position by means of clips to an upright pole. The whole was enclosed within two bell-jars set up as shown in the figure, and at the moment of closing up the apparatus a Petri dish containing

TEXT-FIG. 1.

a number of stromata which had not yet started to puff was placed on a small stand at the bottom of the inverted bell-jar. This dish also contained a number of round cover-glasses coated with thin gelatin. The whole apparatus is air-tight and the stop-cocks at the top and bottom are closed. After a week's interval the apparatus was dismantled, the upper bell-jar being cautiously removed, together with the pole and slides, in order to avoid creating air-currents. Both surfaces of the slides were then examined, but no spores were found on either surface. Spores were, however, abundant on the cover-glasses which had been placed in the Petri dish along with the stromata, thus showing that the latter were functional. The experiment was repeated with the same precautions, but with the two stop-

cocks opened and the upper one connected to a suction-pump. After one hour the coated slides were examined, and the upper surfaces of the slides were found thickly covered with ascospores, but the lower surfaces were quite free of the spores. It was possible, however, that the conditions of the experiment were such that an upward-drawn current working in an enclosed space set up quiescent cushions of air immediately below the slides, which the spores would avoid, and that having been conveyed to the domed wall of the bell-jar they simply gravitated and fell on to the slides. The bell-jars were accordingly discarded and the simpler expedient tried of suspending the pole and slides, placing the Petri dish and contents on the floor, and fanning to create air-currents. The slides again showed the spores exclusively on the upper surfaces. The results of these two experiments seemed to point to the unexpected conclusion that leaf infection might be brought about through the spores falling on the upper leaf-surfaces, and not through the stomata which are found exclusively on the under surface. Inoculation experiments were carried out both in the open air and in the laboratory, in the latter case with branches which had been cut under water. A leaf for inoculation was first sprayed over with rain-water, and during the process of inoculation was partly protected between the parts of a Petri dish. Inoculation was carried out on both leaf surfaces. Leaves that had been inoculated at the same time were left on the tree for varying periods and successively removed, fixed, and microtomed. Though the leaves that had been inoculated on the upper surface showed the cuticle greatly damaged and the epidermal cells in places broken into, no signs of fungus mycelium were detected. Search for evidence of penetration at the lower surface was equally fruitless, and it is worthy of note that the lower epidermal cells showed no signs of injury. These operations, of course, involve an enormous amount of microtoming, and it is possible that the actual points of penetration may have been missed. In view of recent observations of penetration of the cuticle by fungal hyphae, one need not conclude that the thick cuticle and absence of stomata on the upper surface make penetration impossible. Miss E. J. Fry (16) has recently stated that the gelatinous exudate from the rhizines of lichen thalli plays an important part in breaking down the solid substratum when exposed to alternate conditions of dampness and dryness, and it is conceivable that the gelatinous sheath of the ascospore may similarly function in breaking through the cuticle. This would open an interesting field for further study.

METHODS OF INVESTIGATION.

Material was conveyed from the open and immediately fixed; acetic-alcohol of various proportions, Bouin's fixative, and Flemming's weak and strong fluids were employed. The best fixation was, however, observed

when Flemming's strong mixture was diluted with an equal volume of water. This was only slightly superior to a mixture of five parts of absolute alcohol to one of acetic acid, which had the advantage of rapidly penetrating the coriaceous material which forms such a thick covering to the stroma. When the Flemming's fluid was employed it was found necessary, in order to ensure rapid penetration, to dip a whole stroma for a few seconds into warm 30 per cent. alcohol (Digby), and the material was quickly broken up into very small pieces, immediately dropped into the fixative, and the vessel placed for a brief period under an exhaust pump, the material remaining in the fluid for twenty-four hours. Material embedded in wax (52° m.p.) was cut to the thickness of 2 μ for the pycnidium stage, for the apothecium stage 4 μ, and for the cytology of the ascus 5 μ. For nuclear structures the sections were stained with Heidenhain's iron-alum-haematoxylin, followed by a 1 per cent. solution of Congo-red in water, which affords an effective contrast stain for the mycelium. The preparations faded rapidly when mounted in balsam, but when Blackman's method (2) of adding ammonia to the Congo-red was employed, together with mounting in dammar, the effect of the staining was greatly improved. The sections fixed in Flemming's fluid were allowed to remain in this stain for twenty minutes, those from acetic-alcohol for about two minutes, and in each case strong spirit was employed in the washing process. Effective counter-stains were also obtained with haematoxylin, followed by erythrosin or light-green dissolved in clove-oil (Fraser). For staining of the nuclei in the conidia, ascospores, and germ-tubes acetic-carmine was very effective.

The Ascospores and their Germination.

For the microscopic examination and measurement of the ascospores a puffing apothecium was taken and the spores allowed to fall into a Petri dish containing Flemming's weak fixative diluted with ten times its volume of distilled water. After repeated experiments two minutes were found sufficient to effect fixation. A clean slide was now taken, to the centre of which was fixed a small stick of plasticene serving as a handle whereby the slide could be brought in contact with the surface of the fixing fluid and so lift up a film of the fluid containing the floating spores. A large number of spores were thus conveyed to the slide, and though no fixative was employed there still remained, despite the process of washing in repeated changes of water, a large number attached, which again proves the efficiency of the gelatinous sheath as an adhesive agent. The slides were stained with iron-alum-haematoxylin in the usual way, the alum bath being allowed to act for thirty minutes. When the slides were examined from the haematoxylin preparatory to destaining, the gelatinous sheath was very evident, forming a broad hyaline area around the dark spore (Pl. IV, Fig. 3). The filiform

spores were seen to be blunted at one end, narrowing almost to a point at the other extremity. They showed densely granular contents except for a small elongated portion situated almost exactly in the middle; but at other times this portion was seen nearer the blunted end of the spore and sometimes showed a darker stained dot. This homogeneous portion was no doubt the nucleus, and the darker dot a nucleolus. When the spores were examined in acetic-carmine, which served both as a fixing and staining reagent, the elongated structure was much more evident as it absorbed the stain before the cytoplasm, thus standing out very sharply. The gelatinous sheath is of even thickness around the spore, and is about half its diameter in width. It is, however, much more prominent immediately over the blunted end. The spores vary somewhat in size from 55 to 80 μ by 1·5 to
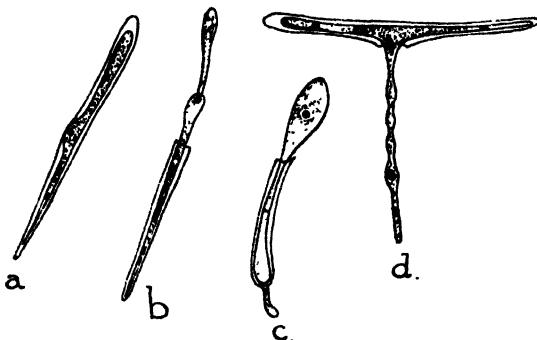
TEXT-FIG. 2. Germination of the ascospores, showing the sheaths broken through by the germ-tubes: (*a*) two-celled spore showing protuberance from the larger cell, to form a germ-tube; (*b*) germ-tube growing directly from blunt end of spore; (*c*) spore forming two germ-tubes; (*d*) a three-celled spore showing germ-tube in a lateral position. × 1,000.

4 μ. When fixed immediately after their discharge in the manner described they were almost invariably found to be unicellular, and very rarely were there seen any which were two- or three-celled.

Germination of the spores was first attempted in distilled water, and if such spores were fixed after a short incubation of five minutes, a large majority were found to be furnished with a cross-wall cutting off a smaller cell at the narrow end. The nucleus in this end was very small, but much clearer in outline than that in the larger cell. In the latter the cytoplasm was abundant and granular, in great contrast with the contents of the smaller cell, where there was usually very little cytoplasm. Germination did not proceed very actively in distilled water, but the first evidence of it was the development of a protuberance in a lateral position, usually from the larger cell (Text-fig. 2, *a*). This swelling then broke through the gelatinous sheath and developed into a germ-tube. The sporelings made very little progress in distilled water, and accordingly various culture media were experimented with. In the first instance a weak decoction of sycamore

leaves was prepared, filtered, and sterilized, but the spores showed little, if any, signs of germination. Some leaves were then cut up and with a little distilled water crushed in a mortar and the fluid filtered. This medium proved more favourable, but very soon became turbid, so that examination of the sporelings was made very difficult. A decoction of cow-dung proved quite ineffective. Finally the sporelings made good progress in a weak infusion of prunes (three prunes to a litre of water) or prune-agar. The usual method of germination was as previously stated, the formation of a germ-tube from the blunted half of the spore, rarely from the narrow half, but sometimes the tube developed from the blunted extremity of the spore
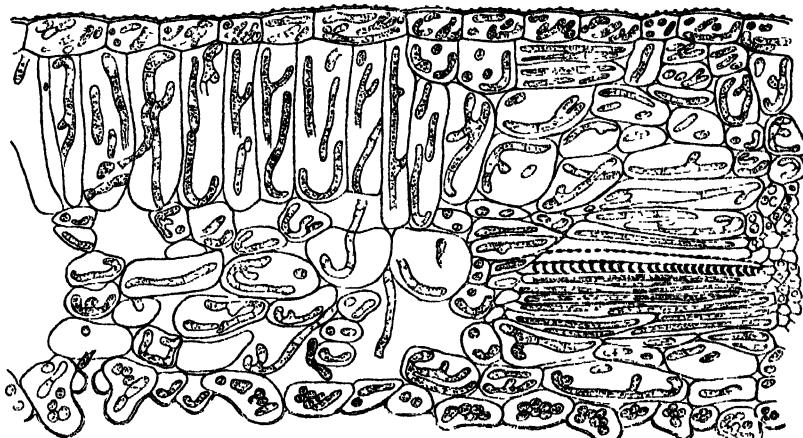
TEXT-FIG. 3. Transverse section of host leaf, showing the mycelium in the tissues at a very early stage of infection when the infected spot is yellow. × 1,000.

(Text-fig. 2, *b*). It remained as a single tube for a considerable length and very few instances of branching were seen, and further examination was rendered impossible owing to growth of bacteria. The germ-tube was sometimes attenuated at its apex, at other times club-shaped. It soon became multinucleate, with the cytoplasm very granular and vacuolate. Its progress in the culture medium was probably not sufficiently advanced for the formation of cross-walls. The sporelings were fixed and stained in the same way as was adopted for examination of the spores, and the nuclei, though small, were well marked. Text-fig. 2, *c*, shows a fairly large nuclear cavity with a distinct nucleolus.

THE MYCELIUM IN THE HOST.

Having gained entrance into the leaves the mycelium proceeds to occupy the cells, and its hyphae can also be seen traversing the intercellular spaces of the spongy parenchyma (Text-fig. 3). At this early stage the

infected area is a small greenish-yellow spot, seen to better advantage when the leaf is held up to the light. The yellow area very soon becomes speckled over with black dots. The hyphae in the cells are seen to follow the contour of the host cells, penetrating into the cytoplasm, which thereby assumes a greatly vacuolated appearance. The host nuclei and plastids remain intact for a considerable time. The septate hyphae consist of regular rectangular cells and are uninucleate. The nuclei are very small, consisting of a prominent nucleolus within a homogeneous nuclear space bounded by a delicate membrane. It is very difficult to detect the presence of chromatin. The cells have scanty vacuolate cytoplasm, and the nucleus is usually situated at the centre. The mycelium also enters the tissues of the vascular bundles, but the water-conducting elements (Text-fig. 3) remain unoccupied until a very late stage in the history of the fungus. The mycelium, in addition to traversing the intercellular spaces, also closely invests the external walls of the spongy cells, but no evidence was observed of the presence of haustoria. A hypha can frequently be seen in one cell seemingly continued into a neighbouring cell, and there is no doubt that the hyphae can grow from cell to cell by penetration of the walls.

Within the cells of the upper epidermis the growth of the mycelium is very active, much more so than in the mesophyll and lower epidermis ; but ultimately these tissues also become densely filled with the fungus. The appearance of the blackening substance, which forms such a prominent feature in the structure of a stroma, takes place at a very early stage in the infected host cells. It first appears within the cells of the upper epidermis, where it forms a fairly thick layer on the inner surface of the outer walls of this tissue (Text-fig. 5), and its rapid deposition in this part accounts for the short time taken for the previously yellow infected spots to turn black. The black substance is also deposited between the cells composing the thickened roof of the apothecia (described below), and it appears again between the hyphae which go to form the floor of these structures. At a later period, but before leaf-fall, it also occurs in the mesophyll tissues between the hyphae.

It is worthy of note that the cells of the lower epidermis, which normally project as tiny utricles, are seen in the infected spots to stand out much more prominently and to be completely filled with mycelium. Whether such prominence is due to further growth of the outer wall of these cells as a result of stimulation by the fungus, or to mere increase in volume of the cell, could not be determined. Though the blackened roof of a stroma is permeable to water, it is possible that the cells of the lower epidermis may be adapted in the manner described to provide for increased absorption at a time when the stromata lie on the surface of the soil and are no longer dependent on the host for water.

The stromata on the fallen leaves are incapable of withstanding much

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desiccation, and it is useless attempting to revive any material (by placing in a moist chamber) which has been constantly wind-swept or heaped up in dry places. Moisture is essential to successful hibernation. If an infected tree is growing in an exposed situation it is often a difficult task to find any stromata on the ground in its vicinity at the time of spore-discharge in early summer, and when these are discovered, they are always found firmly embedded in the moist soil or humus. In sheltered situations the trees are heavily infected, and one can always collect abundant material of well-developed stromata in such a place as the leeward side of a hedge, or where there is a thick carpet of leaf-mould.

DEVELOPMENT OF PYCNIDIA AND CONIDIA.

(*Melasmia acerina*, Levielle.)

As previously stated, the mycelium grows rapidly within the upper epidermal cells of the host leaf, and the development, firstly of pycnidia, and later the initiation of the apothecia, will take place entirely within this tissue before the tree casts off its leaves. A transverse section of an infected spot in which pycnidia are about to be formed (Text-fig. 4) will show the mycelium rather densely packed in the upper epidermal cells, in the vertical walls of which there will soon become evident gaps (Text-fig. 5) setting up lateral communication between these cells. These gaps are formed by the rupture of the vertical walls of the epidermal cells, and this process occurs in these walls at points situated about a fourth of their heights from the outer walls. The hyphae can be seen to impinge closely on the vertical walls at the points where rupture will eventually take place (Text-fig. 4). It is probable that the hyphal tips exert a solvent action on the cell walls at these points, and where this process is extended throughout a specified area in the infected spot there is gradually formed, owing to continual growth of the mycelium within the now ruptured epidermis, a slightly dome-shaped area in the spot. A vertical section of the stroma at this stage shows that the mycelium is resolving itself into distinct zones (Text-fig. 5)—a compact mass of hyphae enclosed in the basal portion of the ruptured epidermis, and a loose mass of hyphae immediately above. The rupture in the host cells gradually widens, the roof increases in convexity, and the upper zone of loose hyphae forms a definite layer of vertical hyphae. The latter are the conidiophores (Text-fig. 6). The external appearance of the infected spot at this time is that of a definite blackened area fringed with a yellowish-green border in which the black pigment appears as a fine granulation gradually shading into the green colour of the leaf. The blotch has now assumed the appearance of a stroma. The pycnidia are, therefore, formed entirely within specified areas of the epidermis. the ruptured cells of which function as a floor and roof.

The conidiophore is at first an elongated cell which soon becomes septated into a short basal cell and an upper, longer, somewhat club-shaped portion—the true conidiophore (Pl. IV, Fig. 1). It shows a very prominent nucleus and nucleolus, together with a small amount of chromatin, and altogether exhibits a much better defined structure than that shown by the vegetative nuclei. In the dilated portion of the conidiophore there is
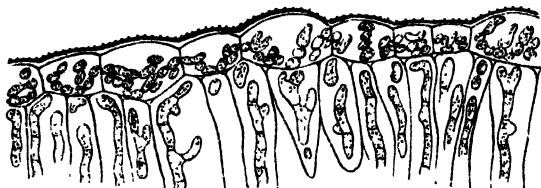
TEXT-FIG. 4. Transverse section of a portion of the host leaf showing the upper epidermis and palisade mesophyll at an early stage of infection. The tips of some of the hyphae in the epidermis are impinging on the vertical walls of these cells. × 1,000.
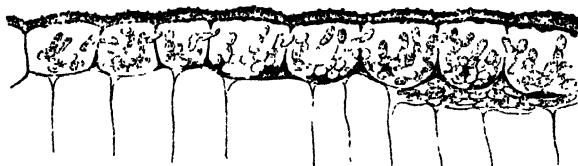
TEXT-FIG. 5. Rupture of the vertical walls of the epidermal cells. Early stage in formation of a pycnidium; deposition of the black substance on the inner walls of the 'roof' and on the 'floor' of the future pycnidium. × 1,000.
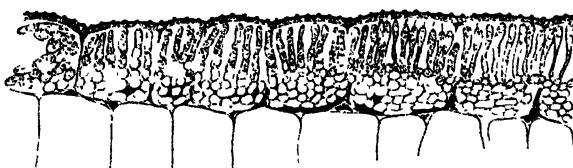
TEXT-FIG. 6. Transverse section of a pycnidium showing the mycelium differentiated into basal plectenchyma and vertical conidiophores. × 1,000.

a quantity of vacuolated cytoplasm, but towards the base the protoplasm resolves itself into a densely granular strand. The apex of the conidiophore grows out into a narrow beak (Pl. IV, Fig. 1), into which the cytoplasm passes as a delicate strand, and at this point there is probably nuclear division, but owing to the extreme attenuation of the beak neither this nor the presence of a second nucleus could be detected. The beak is cut off from the conidiophore and becomes a conidium (pycnidiospore). The same conidiophore may abstrict a number of conidia, but it is extremely difficult to be certain on this point as the conidia are formed in such enormous

numbers, forming dense masses in the section. However, some conidio-
phores grow to such extraordinary length that they were seen to be multi-
nucleate, but their apices showed the same appearance as those of the
shorter conidiophores. Here was probably a provision for the rapid forma-
tion of chains of conidia. A few examples were seen where these long
structures had given rise to conidia in a lateral position.

During the formation of the conidia certain changes have taken place
in the fungal tissue at the boundary of the pycnidium. The mycelium at
the base of the conidiophores has now become interwoven to form a definite
plectenchyma and has extended itself into the neighbouring epidermal cells,
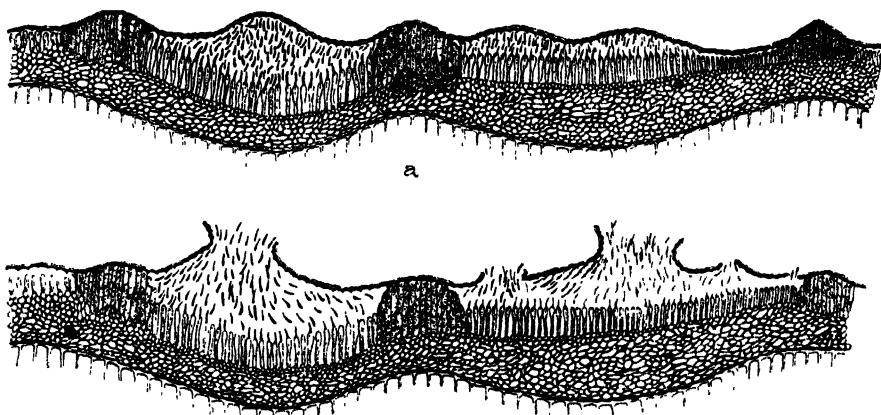
TEXT-FIG. 7. (*a*) Pycnidia in transverse section, showing the 'rampart' tissues or pillars
lifting the roof. (*b*) Rupture of the pycnidia and escape of conidia. × about 2,000 (somewhat
diagrammatic).

these being ruptured in the same manner as described above. At the limits
of a pycnidium it is found that the basal plectenchyma is gradually building
up around the pycnidium a roughly circular rampart made up of concrescent
vertical strands resembling the conidiophores, but with increased septation
(Text-fig. 7, *a*). This is no doubt an adaptation for raising the pycnidium
roof in order to accommodate increased production of conidia. Finally the
roof is broken through, probably by pressure of the conidial mass below
(Text-fig. 7, *b*). In a small pycnidium this opening is usually at the centre,
whereas in larger pycnidia there may be two or more openings. In addi-
tion to the rampart, there are also formed solid pillars of tissue lifting up
the roof in a tent fashion. In some cases the conidial mass will lift the
roof to such an extent that the latter will tear itself away from one or more
of the supporting pillars, to the tops of which are now attached small discs
torn from the roof. Such pycnidia would then have distinct orifices or

ostioles, and this is probably the stage which has been so beautifully figured by Tulasne (34). Finally the pillars themselves may form on their flanks conidiophores and conidia.

The external appearance of the stroma at this stage shows a variable number of pycnidia situated in a group at the centre (Text-fig. 8). They are never found towards the margin of the stroma. At this period the openings or ostioles of the pycnidia are seen to be occupied by a viscid exudate, which can be drawn out with a needle into a short strand of matter which when examined in water dissolves, liberating myriads of conidia. In the stained microtome sections there are also indications of a stringy, structureless mass in which the conidia are embedded. This, again, is probably the exudate above mentioned.

For detailed examination of the conidia the same methods of fixing and staining were employed as were used in the case of the spores. They are straight at the time of formation, but later become club-shaped, often slightly curved and hyaline, measuring about $6\,\mu \times 1\,\mu$. At the blunted end is a dark-stained oval body occupying its entire width; this presumably is

TEXT-FIG. 8. A pycnidial stroma.

a nucleus (Pl. IV, Fig. 2). Repeated attempts to germinate the conidia in culture media failed, and no marks of infection could be observed on the leaves when inoculated with them.

From the structure of the conidia, their failure to germinate and to promote infection, one may probably conclude that they are degenerate bodies of the nature of male cells. These details, together with their cytological characters, agree very closely with the descriptions given by Blackman (1 and 4) of the spermatia of the Uredineae and *Polystigma*, and also by F. T. Brooks (5) of similar structures found by him in *Gnomonia erythrostoma*. The observations also agree with the researches of these authors in the fact that the pycnidia are always formed before the appearance of the archicarps.

In early October one can still find abundant conidia, but certain changes now set in which lead up to the closure of the pycnidia. Some of the latter, relatively few, may, however, be converted into apothecia. After the disappearance of the conidia the torn roof of the pycnidium collapses and comes to lie in close contact with the tips of the conidiophores which still remain. The basal mycelium in the pycnidium is now seen to push up new hyphae between the conidiophores, and these, together with hyphae given out

laterally from the supporting rampart or pillars, proceed to form a thin plectenchyma over the conidiophores. In this region there is later considerable formation of the black material which closely cements the torn roof to the tissues below; this substance also appears between the conidiophores, and particularly in the rampart or pillars, so that the whole mass becomes concrescent. In other cases where the conidia were never discharged, the new plectenchyma is formed in the same way and within it the imprisoned conidia become enmeshed. In cases where the pycnidia are to be converted into apothecia one may see the plectenchyma tissue at the base renewing its

TEXT-FIG. 9. (*a*) A stroma showing apothecia at the margin; in the centre are shown pycnidia probably converted into apothecia; the central dotted area consists largely of pycnidia which have closed up. (*b*) Portion of an apothecial stroma showing rupture of apothecia at spore-discharge. (*c*) Apothecia fully open, showing the ruptured roof reflexed.

growth and sending up fresh hyphae (which later function as vegetative mycelium), which push their way between the conidiophores, in which case the latter remain at the base of the apothecium; or this tissue may carry the conidiophores right up to the roof, to which, with the aid of the black substance, they ultimately become fused. Additional changes take place which will be described in connexion with the formation of typical apothecia.

DEVELOPMENT OF THE APOTHECIA.

The external appearance of a stroma towards early October (but the time varies) shows considerable wrinkling, particularly towards the margin, the central area being now somewhat sunken. With continued exposure to damp conditions (such as were experienced in the autumn of 1923) the wrinkles rapidly become more prominent and resolve themselves into sausage-shaped areas, some straight or curved and others anastomosed into the shape of a Y or X and other fantastic forms (Text-fig. 9).

A vertical section of a stroma showing the early formation of apothecia

exhibits similar features to those which were observed in the development of pycnidia, and the epidermal cells are ruptured in a similar manner (Text-fig. 10). Here, however, the mycelium is much more compacted and completely fills up the host cells. Despite the density of growth one can make out three definite zones—a median zone of hyphae arranged comparatively loosely, and a zone above and another below, both of compact tissue. The upper zone of cells becomes closely adherent to the roof and rapidly develops the black substance which effectively cements its elements together. This substance is also found in the basal zone, but here the depth of the blackened layer is much less. This differentiation into zones is limited to the regions
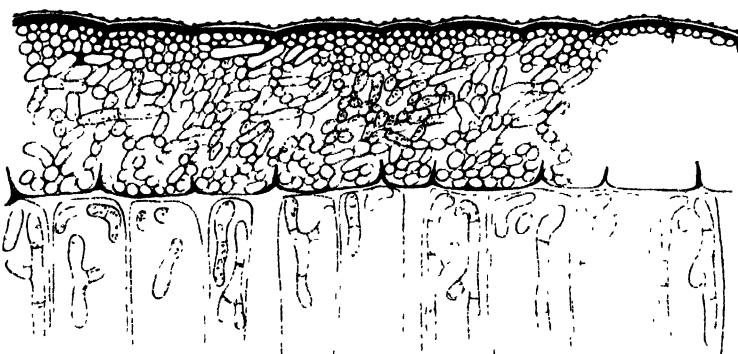
TEXT-FIG. 10. Transverse section of the ruptured epidermis in a situation about to form an apothecium. × 1,000. The mycelium shows three zones—an upper and a lower plectenchyma separated by loose tissue constituting the vegetative mycelium.

which at a later stage will become the cavities of the apothecia. The tissues outside the cavities are continuous throughout the depth of the stroma. The middle zones of loose hyphae in the cavities constitute the vegetative mycelium of the apothecia; the latter are therefore completely enclosed within boundary walls of solid tissue (Text-fig. 11). An apothecium is accordingly protected by a roof consisting of the upper half of the ruptured epidermis, together with the upper zone of blackened and empty cells above described. In preparation for wintering this becomes further thickened on the inner side by additional cells deposited by the loose vegetative hyphae, which are already in contact with the upper zone of cells. The vegetative mycelium now grows actively, some of its hyphae reaching up to the roof, to which they become fixed for a considerable part of their length (Text-fig. 11). Other hyphae, again, seem to abstrict cells at their apices which later become firmly attached to the roof, and as the result of the upward growth of the boundary tissues breaking away from the hyphae which gave rise to them. It can sometimes be seen that the growth of the peripheral tissues is so active that the cells abstricted from

the vegetative hyphae within the apothecia have failed to keep pace with the lifting of the roof, so that these cells remain suspended like beads linked together by delicate strands of some substance which appears homogeneous.
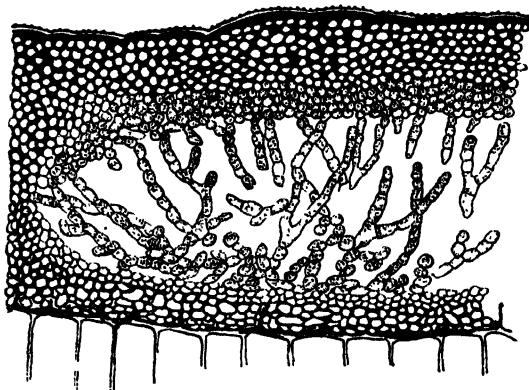
TEXT-FIG. 11. Transverse section of a portion of an apothecium showing solid boundary tissues at the left, and the mode of roof-thickening by additions to the upper plectenchyma from the vegetative mycelium. Appearance of black substance at base of hypothecium. × 1,000.
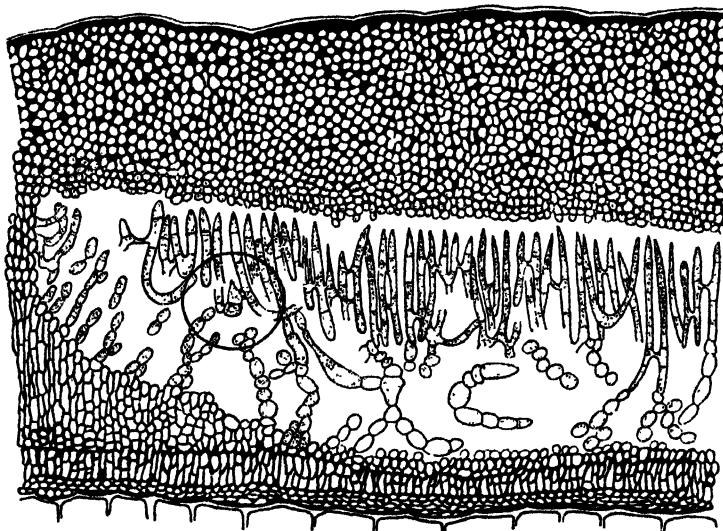
TEXT-FIG. 12. Transverse section of an apothecium showing process of roof-thickening complete. The vegetative hyphae, now erect, are frequently seen linked together by forming H-connexions ; they have also developed attenuated tips simulating paraphyses. (In the small circle is shown the position of a young archicarp drawn in detail on Plate IV, Fig. 5.) × 1,000.

Finally, these additions to the roof form a dense plectenchyma which gradually assumes the blackened appearance seen in the older part. Some half-dozen of the innermost layers, however, retain their cytoplasmic

contents until the rupture of the apothecium. The process above described goes on for a considerable time until the roof approximates in thickness to the depth of the apothecial chamber. It is definitely completed when the apical cells of the vegetative hyphae, by division, have formed pointed terminal cells (Text-fig. 12). These pointed hyphae are later indistinguishable from the paraphyses, and they now stand more or less erect, many of them becoming linked together by forming well-marked H-connexions which establish protoplasmic continuity between these hyphae. They stand out somewhat sharply from the loose tissue supporting them, and the latter may henceforth be referred to as the hypothecium. It is at this stage that the rudiments of the archicarps are discovered.

DEVELOPMENT OF THE ARCHICARPS.

The archicarps, of which there are several in an apothecium, arise as lateral branches, either from the base of the vertical vegetative hyphae or at a somewhat lower level, from the hypothecial cells. They vary greatly in size and general shape, but they can be distinguished from the surrounding vegetative cells by their larger size, denser protoplasmic contents, and multinucleate condition. Some hypothecial cells, however, can be seen to have two nuclei ; but owing to their similarity in size and shape to contiguous uninucleate hypothecial cells one could not conclude with certainty that they developed into archicarps. It is only when the archicarps consist of two or three cells that they can be distinguished from the vegetative cells. A branch destined to become an archicarp may consist of from two to five cells ; and in the latter condition may show great diversity of form, frequently becoming bent or coiled. In archicarps consisting of two or three cells, a large cell can be recognized attached to a small cell below (Text-fig. 13, *a*). In the three- or four-celled condition (Text-fig. 13, *b*, *c*) the large cell is again conspicuous, but now bears one or two cells (Text-fig. 13, *c*) immediately above; in the latter figure there is shown, in addition, a smaller cell in a lateral position. When the archicarp is made up of five cells (Text-figs. 14 and 15; Pl. IV, Fig. 6) the structure is always bent or coiled, and here, again, a larger cell stands out conspicuously from the others. Taking all these forms together, one may conclude that the larger cell is an oogonium (ascogonium), the smaller cell immediately below or at its side and in connexion with the parent hypha a stalk cell, and the remaining cells, of variable number, are a 'trichogyne'. The apical cell of the trichogyne is frequently pointed and prolonged into a small beak (Text-fig. 13, *c*, and Pl. IV, Fig. 5), which stains deeply with Congo-red. No structure of the nature of an antheridium was seen. It has been mentioned above that cells of the vegetative tissue are in protoplasmic continuity with each other by virtue of H-connexions. In few instances only has the

oogonial cell or a cell of the trichogyne been seen connected with contiguous cells in a similar manner (Pl. IV, Fig. 5). Such a phenomenon, when it occurred, seemed highly suggestive of the presence of an antheridium[1] with a fertilizing tube, but when two such connexions were seen to pass over from one and the same cell in an archicarp, one to each of two neighbouring cells of a vegetative hypha (Pl. IV, Fig. 5), it resolved itself into a case of mere linkage of an element of the archicarp with adjacent vegetative cells. Such H-connexions never assumed the diameter of the large open pores to be described below; they were merely tubes of extreme narrowness, identical with those occurring between the vegetative hyphae.

At the earliest stage in the history of the archicarp the protoplasm of
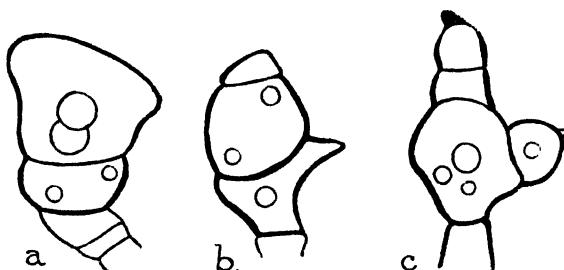
Text-fig. 13. Young archicarps. × 4,000. *a*) Showing large oogonium and stalk cell below; the 'trichogyne' cells have not yet been formed. The rings represent small refringent spheres, probably of food reserve. (*b*) Oogonium furnished with a one-celled 'trichogyne'; stalk cell probably about to put out H-connexion. (*c*) Oogonium with lateral cell putting out H-connexion; two-celled trichogyne with 'beak'.

its constituent cells is denser than that in the vegetative cells and contains a few large vacuoles. The oogonial cell invariably, and frequently the other cells as well, contains a number of spherical, highly refringent bodies, which show no affinity for stains (Pl. IV, Figs. 5 and 13). These bodies are of very rare occurrence in the vegetative cells. They are found in the archicarps only at these early stages and disappear entirely in the older structures. They are evidently of the nature of food reserve, and are probably of the same composition as the bodies seen by Cutting (6) in the archicarp cells of *Ascophanus carneus*. The nuclei in young archicarp cells are very small and show no difference in size from the vegetative nuclei. They show a well-marked nucleolus surrounded by a homogeneous space bounded by a nuclear membrane. While the stalk cell and the cells of the trichogyne are often seen to be uninucleate, this condition has never been seen in the oogonial cell. In this cell the nuclei vary from two to six, but no cell of the archicarp at this early stage contains numerous nuclei.

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[1] The writer pointed out in his paper given at the Liverpool meeting of the British Association that the presence of an antheridium was probable, but the investigation of the archicarps was at that time incomplete.

The cross-walls between the cells of the archicarp now break down, forming wide pores, thus setting into communication all its constituent cells with the exception of the stalk cell (Pl. IV, Figs. 4, 6; and Text-fig. 14). The spherical granules which have been so frequently described in other fungi were only seen in a few instances, and the formation of 'pads', such as occur in *Ascophanus* (Cutting) and other types, were not observed at this stage, or later, in connexion with the development of these pores. By this time the nuclei in all the cells have increased in number but diminished in size.
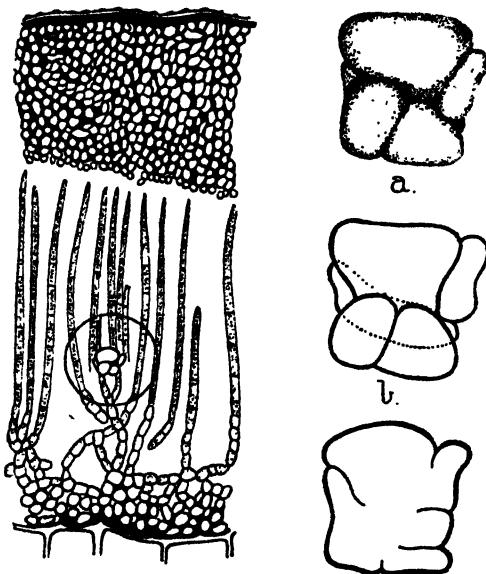
Text-fig. 14. Portion of a transverse section of apothecium showing the relative position of a mature coiled archicarp of five cells. × 1,000. (*a*) The archicarp shown enclosed in ring, enlarged × 4,000; the largest cell is the oogonium. (*b*) The same showing obscured cell in focus. (*c*) The same focused to show all cells of the coil in communication by break-down of their intervening walls.

They are seen in pairs, presumably as a result of division, the details of which could not be determined on account of the small size of the nuclei. It is now seen that the archicarp has resolved itself into a scolecite of a form similar to that seen by Welsford (35) in *Ascobolus furfuraceus* ; Ramlow (30) in *Ascobolus immersus*, and Schweizer (32) in *Ascobolus citrinus*. The mode of formation of these wide pores has not been determined, and in the absence of any structures resembling 'granules' or 'pads', it may perhaps be brought about by solvent action of a nature similar to that which preceded the rupture of the epidermal cells.

FERTILIZATION.

Communication having thus been established between the cells of the archicarp, there now takes place a migration into the large oogonial cell of the contents of the remaining cells (with the exception of the stalk cell, which is not put into communication with the others) (Pl. IV, Figs. 4, 6). The extent of this discharge of contents by the cells in connexion with the oogonium is somewhat variable. In Pl. IV, Fig. 4, is shown an archicarp in which the cross-walls (seen obliquely) are now mere rings, and here there is complete protoplasmic continuity between the large irregular oogonium and the two cells above it, but not as yet with the terminal cell ; the transverse wall at this point, however, was very thin, and it is probable that the pores are not formed simultaneously. Text-fig. 16, *a* and *b*, represents another archicarp in different foci which shows very wide pores. In Pl. IV, Fig. 6, a curved archicarp is seen in which a terminal cell has seemingly retained its contents. In this case no communicating pore could be seen, and this may be due to the section having passed through another plane. It is possible that a cell may retain at least part of its contents despite the presence of a communicating pore, but very few instances of such a condition have been observed.

As above stated, the nuclei of the constituent archicarp cells increase in number before migration into the oogonium begins, and nuclear division may continue within the oogonium, for instances were seen where this cell contained very numerous nuclei. The nuclei aggregate at the centre of the oogonium (ascogonium), forming a somewhat close mass (Pl. IV, Fig. 6) ; and on account of their small size it is exceedingly difficult to determine, even approximately, the number of nuclei. Where they are seen in fewer numbers towards the periphery of the ascogonium, the nuclei are in close contact, very frequently in pairs. No evidence was seen of nuclear fusion in the ascogonium. In the ascogenous hyphae the nuclei are also found in pairs (Pl. IV, Figs. 6, 8, and 17), a condition seen by Claussen (7) in *Pyronema*, Ramlow (30) in *Ascobolus immersus*, and Schweizer (32) in *Ascobolus citrinus*.

THE ASCOGENOUS HYPHAE.

From a careful study of such figures as those shown in Pl. IV, Fig. 6, Text-figs. 16, *c*, and 17, where the ascogonium is directly seen and not obscured by other archicarp elements, one has come to a definite conclusion that the only cell of an archicarp to give rise to ascogenous hyphae is the oogonium. This conclusion agrees with the observations of Welsford (35) on *Ascobolus furfuraceus*, Harper (19) on *Pyronema*, Blackman and Fraser (3) on *Humaria granulata*, and Schweizer (32) on *Ascobolus citrinus*. The

ascogenous hyphae begin to appear simultaneously with, or very soon after, the formation of the pores in the archicarp. The three diagrams in Text-fig. 16, *a*, *b*, *c*, are of interest in that they show three important stages seen in two neighbouring sections passing through the same archicarp. *a* and *b* represent the archicarp in different foci ; *c* is the next section, which has evidently passed through the larger ascogonial cell only, here seen producing ascogenous hyphae, the terminations of which could not be seen in the section.

In only a few instances (one of which is figured on Pl. IV, Fig. 6) has an ascogenous hypha, whilst still in direct communication with the ascogonium, been seen to remain un-branched and form the customary hook of a terminal and penultimate cell. The ascogenous hyphae almost invariably branch repeatedly in a most intricate manner, and their study in the shallow apothecia has been a matter of great difficulty. Concurrent with their ap-pearance, or somewhat earlier, are found the paraphyses growing from the hypothecial cells, and in rather closer arrangement around the ascogonia below which they arise, some from the stalk cells, others from the hypothecium (Text-fig. 15). At this stage, and up to the rupture of the apothecium, the paraphyses are perfectly straight and regular.

The writer is strongly of opinion that the archicarps of *Rhytisma acerinum* very frequently send out branches (Text-fig. 18), (presumably from the ascogonial cell, but of this one cannot be certain) which develop into new or ' secondary' archicarps, a pheno-

TEXT-FIG. 15. Portion of a transverse section of an apothecium which had previously functioned as a pycnidium. × 1,000. Note the vertical concrescent conidiophores at base of hypothecial cells. In the circle is shown the relative position of an archicarp of five cells, which is drawn in detail on Plate IV, Fig. 6. × 1,000.

menon which has been seen by Ternetz (33) in *Ascophanus carneus*. Frequent evidences are seen of archicarps putting out ascogenous hyphae which can be seen growing for some distance, obliquely or laterally, and bearing, seemingly at their extremities, intricate coils of multinucleate cells, from which again arise ascogenous hyphae, and finally young asci (Text-fig. 18). The ascogenous hyphae at their early formation are rich in contents and multinucleate, but they very soon become septated into short cells which are binucleate (Pl. IV, Fig. 8). Some of the hyphae extend laterally for a considerable distance before finally forming, in the manner described below, asci which at first arise in groups. The

intermediate cells soon lose their contents and their nuclei probably degenerate. It is difficult to detect the presence of hooks in the termination of the ascogenous hyphae, together with the cutting off of the usual terminal and penultimate cell (Pl. IV, Fig. 7). In consequence one concludes that the usually described method of ascus development very rarely occurs; on the contrary, the penultimate cell most frequently
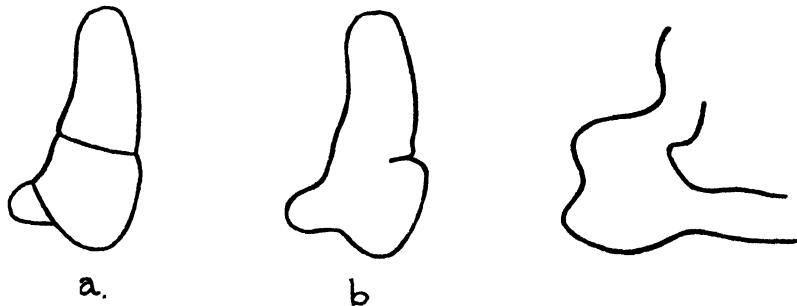
TEXT-FIG. 16. (*a*) Archicarp with large basal oogonium bearing a single-celled elongated 'trichogyne'. (*b*) The same in different focus showing the wide pore between the cells; the oogonium is putting out an ascogenous hypha. (*c*) The same archicarp as it appears in the neighbouring microtome section, now showing the oogonium alone putting out two ascogenous hyphae. × 4,000.

FIG. 17.   FIG. 18.
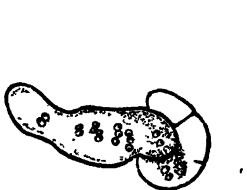
TEXT-FIG. 17. A coiled archicarp showing oogonium with nuclei, some in pairs. The oogonium is forming one wide ascogenous hypha which contains a pair of associated nuclei. Perforation between two cells is shown, and from the appearance of the continuity of cell contents there is evidently communication between the oogonium and the cells on the right. × 2,000.

TEXT-FIG. 18. An archicarp and ascogenous cells with groups of paired nuclei. The archicarp on the right, containing a perforated cross-wall, is probably a '*secondary*' archicarp, where the connexion between it and the archicarp on the left is broken. × 2,000.
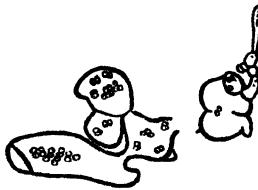
shows a multinucleate condition and gives rise to other ascogenous hyphae. The subsequent history of such hyphae is very difficult to follow, as they usually become more or less horizontal, growing in and out amongst the paraphyses, which now are in such dense array that their examination is rendered increasingly difficult.

A remarkable feature occurs in the formation of the asci, affording a case strikingly parallel with Brooks's (5) observations on *Gnomonia*. The ascogenous cells branch repeatedly, and their paths are difficult to follow on account of the closely interwoven elements of the hypothecium. Where one would expect an ascogenous cell, from its position in the hypo-

thecium, its shape, and comparative length, to form a young ascus, it is found frequently to have even three or four pairs of associated nuclei (Pl. IV, Figs. 7, 8). Such a cell will eventually branch in a most contorted manner, each branch finally containing a pair of nuclei. These branches appear to form asci directly without the intervention of the customary hook and penultimate cells. The pair of nuclei in the branch remain in association for a comparatively long period, and fusion is consequently delayed, and may be frequently seen taking place in well-defined club-shaped asci. The nuclear figures at this stage (Pl. IV, Figs. 9, 10) are strikingly similar to those described by Harper (21) in *Phyllactinia*. The fusing nuclei show the characteristic 'central body' and chromatin sheaf, but it is exceedingly difficult to follow the exact details of the association of the elements forming the chromosomes.

FORMATION OF THE ASCUS.

The belated fusion of the two nuclei to form the definite nucleus of the ascus can only with certainty be made out at a much later stage, when additional ascogenous hyphae have been formed, at which time the asci are arranged in close formation in a definite hymenium. The hypothecium, which up to this stage consisted of a more or less loose tissue, has now become compacted at the base of the apothecium. Here, asci in all stages of development can be seen arising from the ascogenous cells, some with the two nuclei in process of fusion, others uninucleate, together with typical club-shaped asci furnished with a well-marked nucleus, nucleolus, chromatin (described below), and abundant protoplasm.

CYTOLOGY OF THE ASCUS.

As previously stated, the stages in the fusion of the nuclei in the young asci can best be determined from a study of the young asci when they are in close formation. At their first initiation the young asci are somewhat irregular in their direction of growth; in each there can be recognized a pair of nuclei, in which the two nucleoli and chromatin masses can be clearly distinguished, the latter consisting of delicate strands in rather loose arrangement. The first stage in the fusion consists in the apposition of the two chromatin masses, while the two nucleoli remain distinct (Pl. IV, Figs. 9, 10). Each chromatin mass is seen associated with a centrosome. Ultimately the two nucleoli fuse (Pl. IV, Fig. 11). A stage showing the fusion of the centrosomes was not detected. The chromatin, now forming one mass (Pl. IV, Fig. 12), shows the strands arranged roughly parallel. The nucleus then assumes a resting condition in which the nucleolus is well marked, occupying a polar or lateral position in a clear nuclear area.

At a point on the periphery of the nuclear cavity and directly opposite the nucleolus is a small dark body ('central body') to which is attached the darkly stained chromatin in the form of irregular threads spreading out into the nuclear cavity. Some threads are thick and heavily stained; others thin and lightly stained (Pl. IV, Fig. 13). The whole structure again bears a marked resemblance to the resting nucleus in the ascus, as figured by Harper (21) in *Phyllactinia*. (These features are again repeated in the resting nuclei at the two- and four-nucleate (Pl. IV, Figs. 21, 24) stages in the ascus. In the eight-nucleate condition the nuclei are too minute for detailed study of their structure.) The only case of nuclear fusion occurring during the whole life-history is that which is found in the young ascus. The greater part of the protoplasm in the ascus presents an alveolated appearance, but near the apex the protoplasm forms a dense mass, and it is here that the fusion nucleus takes up its position.

First Division in the Ascus.

The fusion nucleus now passes over into synapsis (Pl. IV, Fig. 14), and it is at this stage alone that synaptic contractions are found. This is followed by a stage strongly resembling a spireme, but the presence of a continuous thread could not be definitely established (Pl. IV, Fig. 15). The first nuclear division in the ascus is taken to be the reduction division of the meiotic phase.

The stringy chromatin above described is seen next to have resolved itself into definite independent strands approximately of the same thickness. After repeated examination of several preparations, five such strands could be counted in the nuclear space (Pl. IV, Fig. 16). It seems very probable that in *Rhytisma* there exists the phenomenon of *chromosome association*, similar to that occurring in *Phyllactinia*. From a consideration of the nuclear figures above described, one therefore concludes that the five chromatin strands are really five *bivalent* chromosomes, the association of chromosomes having occurred at some stage subsequent to the fusion of the paired nuclei. The bivalent chromosomes now become typically staple-shaped (Pl. IV, Fig. 17).

The first spindle is intranuclear, but it is very difficult to trace the exact changes leading up to its formation (Pl. IV, Fig. 18). It lies somewhat obliquely at the apex of the ascus, with a centrosome at each pole, from which radiate a few delicate spindle-fibres. The spindle is very small but clear, and at metaphase shows five short oval chromosomes. Typical examples of early or of late anaphase have not been seen at any of the stages of division. The first division seems to be passed through very quickly, and the two daughter nuclei are linked up at the telophase stage

(Pl. IV, Fig. 19) by a kinoplasmic thread. After the reorganization of the two nuclei is complete (Pl. IV, Figs. 20, 21) the chromatin in each nucleus is seen to converge and meet at one point.

Second Division in the Ascus.

The two spindles at this stage are somewhat smaller than the first, but still very clear. They show the same features as were seen in the first division, and here again five chromosomes can be counted at metaphase (Pl. IV, Fig. 22). At telophase (Pl. IV, Fig. 23) the four daughter nuclei are linked up by delicate kinoplasmic fibres. Their reorganization (Pl. IV, Fig. 24) shows again the convergence of the chromatin to a common point.

Third Division in the Ascus.

The four-spindle stage (Pl. IV, Fig. 25) shows spindles of about the same size as those in the second division, and again five chromosomes can be counted at metaphase. The eight nuclei at telophase are again joined by the characteristic threads (Pl. IV, Fig. 26). The reorganized nuclei are exceedingly small, and under very high magnification appear as mere black dots scattered in the cytoplasm of the ascus.

DEVELOPMENT OF THE ASCOSPORES.

The very early changes in the subsequent history of the eight minute nuclei could not be determined. Very soon, however, eight dark stained dots can be seen, each in rather close association with another dark stained mass of irregular shape (Pl. IV, Fig. 27). Later on it becomes clear that each dark dot, now a small sphere, acts as a centre from which radiate a number of straight delicate fibrils with their extremities in close contact with the dark irregular masses, which are now much bigger (Pl. IV, Fig. 27, *a*). These dark spheres may now probably be identified as spherical centrosomes, sending out astral rays (the delicate fibrils) in contact with masses of chromatin (the dark irregular masses). It is not at all clear that the astral rays are here the agents whereby the delimitation of the ascospore is initiated, for there now rapidly follows the formation of well-defined planes of cleavage in the cytoplasm attended by attenuation and elongation of the chromatin towards the base of the ascus (Pl. IV, Fig. 28). The cleavage and vacuolation goes on apace, and the chromatin now becomes an elongated thread furnished with thick dark beads at intervals in its course (Pl. IV, Fig. 29) until finally each of the eight spores is sharply delimited by a thin wall (Pl. IV, Fig. 30). At the extreme apex of the ascus there is seen a protoplasmic mass, which is utilized to form a dome-shaped thickening of the apex of the ascus wall. The asci towards maturity

elongate considerably, and measure about 130 μ × 10 μ. The filiform ascospores are arranged in a fasciculate manner with their blunt ends towards the apex of the ascus. Their nuclei are difficult to define, but the darker stained masses which occupy about a fourth of the entire length of each spore probably consist of chromatin (Pl. IV, Fig. 30). It is only when the germination stages of the spores are studied that their nuclei assume a definite shape (Text-fig. 2). A transverse section of the mature ascus shows the eight spores arranged in a group, a central spore being surrounded by the remaining seven, each spore being furnished with a massive sheath (Pl. IV, Fig. 31).

RUPTURE OF THE APOTHECIUM, AND SPORE-DISCHARGE.

In preparation for the final discharge of the spores, certain changes in the roof of the apothecium are initiated long before the spores themselves are developed. At the time when the uninucleate asci are being formed there is seen in the roof-tissue, in a zone situated exactly over the centre of the apothecium (when examined in a vertical section), a row of about a dozen cells running parallel with the roof, and invariably located about three-fourths of its depth below the cuticle (Text-fig. 19). As previously mentioned, the innermost layers of the roof-tissue, some half-dozen in number, retain their contents, and the row of cells under consideration is situated in this part. The cells in this single row soon lose their contents, and, later, their walls break down and disappear; thus there is formed in the interior of the roof a small hyaline rift. An additional layer of the cells immediately above and below the rift will also undergo a change, which, however, is confined to those of their walls directly abutting on the rift into which they now discharge their contents (Text-fig. 20, *c*). The rift, by expansion, now assumes the dimensions of a small cavity which extends along the whole long axis of the apothecial roof. The process of cell-destruction is not progressive, and is strictly confined to the above limits. The discharged protoplasts soon dissolve and the cavity becomes again a clear space which gradually increases in dimension. Concurrently with the formation of the cavity, the whole apothecial roof is gradually being raised by growth of its boundary tissues, and the floor of the apothecium, previously somewhat level, is now decidedly concave (as seen in vertical section, Text-fig. 20, *b*). In addition, certain layers in the cells of the floor have already developed the black substance on their walls, and now resemble the cells of the roof. The floor is thus well consolidated. By this time the cavity has so increased in vertical diameter that the concave floor of it bulges into the apothecial chamber. Owing to the growth of the boundary tissues referred to above, and the consequent raising of the peripheral portions of the apothecial floor, the paraphyses of the whole apothecium

now impinge on the protruding part of the roof. Moreover, the asci, some of which, at least, have by now formed mature ascospores, are arranged with their axes parallel with the paraphyses and directed towards the concave roof. It is at this stage that the tips of the paraphyses become hooked,
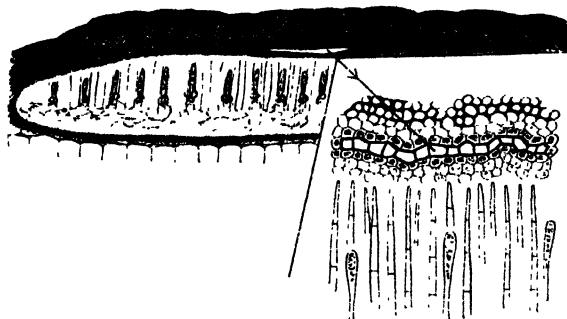
TEXT-FIG. 19. A vertical section of an apothecium showing the formation of a 'rift' in the interior of the roof. In the apothecial chamber are shown a series of archicarps (see Plate IV, Fig. 6). Inset, details of early formation of 'rift' from a single layer of cells, situated in about the fourth layer from inside; the layers of cells abutting on the empty rift-cells show protoplasmic contents.
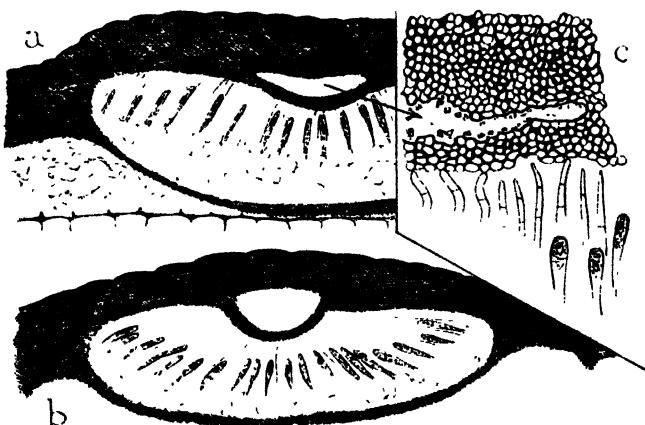
TEXT-FIG. 20. (*a*) Showing expansion in volume of the 'rift' or roof-cavity, and the protrusion of the floor of the roof-cavity into the apothecial chamber, the floor of which is now concave. Inset, (*c*) details showing breaking down of the cells abutting on the 'rift'; tips of paraphyses becoming hooked owing to pressure from protruding roof. (*b*) Showing maximum expansion in volume of roof-cavity, now filled with mucilaginous substance. Paraphyses and asci all directed towards protruding roof.

evidently from pressure exerted on them by the roof. The gradual increase in the volume of the roof-cavity is probably accounted for by the existence within it of mucilaginous or other substance, which is capable of swelling by the absorption of moisture. Further, the formation of the rift in the lower part of the roof, leaving an appreciable thickness intact above,

still renders the thicker part sufficiently rigid to withstand much displace-
ment, and consequently the thinner floor portion is compelled to protrude
into the apothecium.   It is conceivable that the effect of such protrusion is
to increase the pressure within the closed apothecium.   The result of such
a pressure from within will perhaps explain why the roof itself now increases
in convexity, which ultimately brings about its rupture along a median line
immediately above the roof-cavity (Text-fig. 21).   The matrix within the

TEXT-FIG. 21. Rupture of that part of the roof immediately above the roof-cavity and exposure
to the air of the mucilaginous substance. In the apothecial chamber are shown bundles of
ascospores about to be expelled.
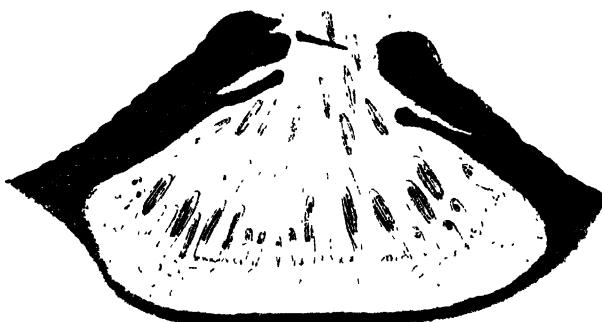
TEXT-FIG. 22. Showing rupture of the inner protruding floor of the roof-cavity and discharge of
ascospores.

space is now in contact with the atmosphere, and constitutes the exudate in
the apothecial fissures previously described.   In dry air the exudate con-
tracts into the fissure (Text-fig. 21).   The result of such contraction will be
that the sagging floor of the chamber in contact with the drying .exudate
will be pulled up and finally ruptured (Text-fig. 22).

The ascospores are discharged intermittently.   As previously stated,
a vertical section of an apothecium at this stage will show asci at all stages
of development, together with asci which have discharged their spores.   The
dehiscence of the asci takes place in an irregular manner, after which process
their walls collapse.   A newly ruptured apothecium shows its asci and

paraphyses all directed obliquely towards the fissure, an adaptation which is brought about, as previously described, by concavity of the apothecial floor. Some apothecia may remain permanently open by narrow fissures only, whilst others may have the broken roof bent back to its utmost limits (Text-figs. 9, *c*, and 23) ; such fully open apothecia expose their asci and paraphyses in a vertical, and not a convergent manner.

TEXT-FIG. 23. A widely open apothecium.

GENERAL CONSIDERATIONS.

There are two possible interpretations of the conidia—either they are male cells or they are asexual cells capable of causing infection. The fact that they appear before formation of the archicarps is a point in favour of the former view. They are exceedingly small and furnished with a well-marked nucleus, thus bearing marked resemblance to the spermatia of the red algae. In addition, the fact that they are liberated from the pycnidia in a viscid fluid renders it possible that they could be conveyed (probably by insects) to the apothecia which invariably surround the pycnidia on the stroma. Moreover, all attempts that have been made to induce them to germinate or to promote infection have failed. Granted, in spite of the fact that no true fertilization has been hitherto observed, that these bodies are functional male cells, it is evident that such fertilization cannot possibly take place except in those apothecia which have been converted from pycnidia. An archicarp is furnished with a number of cells, some of which may constitute a ' trichogyne ', and since there takes place the development of pores which set up communication between its cells and the oogonium, such conditions would be favourable to the fertilization of the oogonium by a spermatium. However, the number of pycnidia in a stroma in comparison with the number of apothecia is exceedingly small ; and, moreover, it is somewhat exceptional to find a pycnidium converted into an apothecium. Most pycnidia become closed up without being succeeded by apothecia. The apothecia of *Rhytisma* arise in great numbers towards the margin of the stroma, quite outside the areas occupied by the pycnidia. Furthermore, the formation of apothecia entirely within the epidermis of the host and the development of a thick continuous roof before the appearance of ascogonial cells at once preclude the possibility of fertilization of

the latter by spermatia (conidia). Such a phenomenon could only be rendered possible if the ' trichogyne ' of the archicarp were enabled to penetrate the roof, and so expose itself for fertilization. No instances, however, were seen where trichogynes ever penetrated the surface of the stroma. It is possible that in ancestral forms of the fungus they were capable of reaching the exterior in the manner evident in many lichen types. Indeed, there are striking features in the structure of *Rhytisma* that are strongly reminiscent of the lichens. These are discussed below.

The absence of nuclear fusion in the ascogonium of *Rhytisma* leads one to conclude that, in this species, the only nuclear fusion which takes place is the one that occurs in the ascus. While much of the published work on the sexuality of the Ascomycetes seems to imply that there are two fusions, other researches (such as those of Blackman on the Uredineae, Claussen on *Pyronema confluens*. Schikorra on *Monascus*, Faull on *Laboulbenia*, Ramlow on *Ascobolus immersus* and *Ascophanus carneus*, Brooks on *Gnomonia erythrostoma*, and quite recently Schweizer on *Ascobolus citrinus*) indicate that the association of nuclei in pairs must be considered as a reduced type of fertilization followed by delayed fusion in the ascus. All the evidence seems to show that the phenomena observed in *Rhytisma* conform with the types named.

The archicarp of *Rhytisma* shows many features of similarity with several investigated types of Ascomycetes. It bears marked resemblance to the scolecite forms occurring in *Lachnea stercorea* (Fraser (11)), *Ascobolus furfuraceus* (Welsford (35)), *Ascobolus immersus* (Ramlow (30)), *Ascobolus citrinus* (Schweizer (32)), and *Ascophanus carneus* (Cutting (6)). A striking difference, however, exists in that the number of nuclei in the ascogonial cell of *Rhytisma* is at first relatively small. Again, in this species the cells of the archicarp differ in size, and it is probable that only one gives off ascogenous hyphae, thus differing from such types as *Lachnea cretea* (Fraser (15)), *Ascophanus carneus* (Cutting), &c.

The archicarp presents points of strong similarity with that of lichens. It is divisible into three portions, a stalk, an oogonium, and a varying number of cells probably forming a ' trichogyne ', the latter assumption being based on the interpretation that the conidia in phylogeny once functioned as fertilizing spermatia. The mode of formation of the stroma and apothecial roof is strikingly similar to the development of the plectenchymatous cortex covering the thallus of such a type as *Peltigera*. In *Sticta, Parmelia*, &c., where two cortices are produced, this suggests an analogy between such a condition and the reinforced black tissues seen in the roof and floor of the *Rhytisma* apothecium.

The coriaceous consistency of the stroma, together with the development of a roof which may be considered as an epithecium, shows similarity of structure with certain members of the Patellariaceae in which

the fruits are closed at first, but which later open by a narrow or stellate split.

, The systematic position of the genus *Rhytisma* has been discussed by various systematists, and latterly by Hoehnel (22), but most recently the genus has been placed by Professor Dame H. Gwynne-Vaughan (18) in the Phacidiales because of the extent of exposure of the hymenium at maturity, and further, it has been separated from the allied group Hysteriales by a difference in shape of the ascophore and the manner in which it opens. From the present investigation it is seen that the ascophores of *Rhytisma acerinum* assume different shapes, and where they are elongated the rupture takes place by a slit as in the Hysteriales. Most of the apothecia of a stroma are of this shape, and round ascophores opening in a stellate manner are infrequent. Further, cases frequently occur where the hymenium at maturity is as fully exposed as in a typical *Peziza* (Text-figs. 9, *c*, and 23). Taking all the facts into consideration, and particularly the morphology of the ascocarp, it seems justifiable to place *Rhytisma acerinum* in the family Phacidiaceae, along with the Stictaceae in the group Phacidiales, as adopted by Professor Dame Gwynne-Vaughan. Although striking details in the structure of the archicarps have here been brought forward suggesting affinities with the Ascobolaceae, it may still be highly speculative to express an opinion on the phylogeny of the fungi from a discussion on the nature of these organs.

The nuclear phenomena studied in the cytology of the ascus in *Rhytisma acerinum* afford a striking parallel with those in *Phyllactinia*. If the interpretation here is correct, then the deferred fusion and association of chromosomes in the nucleus, and the fact that the same number of chromosomes occurs in the three divisions in the ascus, may be put forward in support of the theory that the process of simple association of nuclei in the oogonium constitutes a reduced fertilization in the sexuality of this fungus.

SUMMARY.

1. *Rhytisma acerinum* is a parasite forming stromata on the leaves of the Sycamore, and after leaf-fall the fungus probably thrives on the food stored chiefly by the mycelium filling up the host cells which are permanently attached to the stroma. The mycelium consists of uninucleate cells.

2. Pycnidia and apothecia are formed on the same stroma.

3. The pycnidia are similar in structure to spermogonia. The conidia are therefore probably spermatia, now considered to be functionless.

4. Apothecia arise later than pycnidia. Pycnidia may be converted into apothecia, but the latter are usually formed *de novo* towards the margin of the stroma.

5. The sexual apparatus consists of a 'scolecite', in which one cell, viz. the ascogonium, is believed to form ascogenous hyphae. There is no antheridium.

6. There is no fusion of nuclei in the ascogonium. The ascogonial nuclei migrate into the ascogenous hyphae, where they are seen arranged in pairs.

7. The only nuclear fusion observed takes place in the ascus.

8. The phenomena of belated fusion and chromosome association are observed in the ascus.

9. Five chromosomes are seen at all three divisions in the ascus. The anaphase stage has not been seen.

10. The dehiscence of the apothecia with subsequent spore-discharge is described.

It is a great pleasure to acknowledge my indebtedness to Professor Lloyd Williams for his help and criticism throughout the progress of this work.

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EXPLANATION OF PLATE IV.

Illustrating Mr. S. G. Jones's paper on *Rhytisma acerinum*.

All figures have been drawn with the aid of the camera lucida. Immersion lens, Zeiss $\frac{1}{12}''$ apochromatic, with oculars 6, 8, 12, and 18.

Fig. 1. Apex of a conidiophore showing nucleus with a mass of chromatin; conidium at apex. × 2,400.

Fig. 2. Conidia. × 2,400.

Fig. 3. Ascospore and sheath; nucleus at centre. × 1,000.

Fig. 4. Archicarp showing large oogonium containing a number of paired nuclei. Stalk cell at left with cross-wall intact. Three 'trichogyne' cells showing two cross-walls appearing as rings and completely perforated. The contents of these two cells are migrating into the oogonium; the cross-wall separating the terminal cell is still intact, but very thin. × 4,000.

Fig. 5. A young archicarp showing large basal oogonium containing five nuclei and a large refringent sphere (ring); its stalk cell is on the left. The 'trichogyne' cell, containing two nuclei, is prolonged into a short, thick beak, and is attached to two cells of a neighbouring vegetative hypha by means of two H-connexions. × 4,000.

Fig. 6. A mature archicarp consisting of a coil of five cells, three of which, in communication by means of pores, are empty except for a small quantity of cytoplasm. The large cell is the oogonium and contains a central mass of nuclei, some of which are paired. The oogonium has formed a vertical ascogenous hypha showing a typical 'hook'; the terminal cell of the hook is uninucleate, but the cytoplasm of the parent hypha is in close contact with its wall and is seemingly about to pass into it another nucleus; the penultimate cell has two pairs of nuclei. The parent hypha is still multinucleate and will probably give rise to lateral branches. × 4,000.

Fig. 7. An archicarp showing internal walls broken down. The ascogenous hypha on the right has formed a terminal hook in which are found two pairs of associated nuclei, but no cross-walls are present dividing the 'hook' into terminal and penultimate cells. The ascogenous hypha on the left is forming a coil of two or three cells which may correspond to a 'hook' or to a stage in the formation of a 'secondary' archicarp. × 2,000.

Fig. 8. A horizontal and septated ascogenous hypha forming, on the left, branching ascogenous hyphae which may again form branches into each of which will pass a pair of nuclei before finally becoming asci—without the intervention of 'hooks'. The object on the immediate right is a dilated and much-branched ascogenous cell probably derived from the hypha below. × 2,000.

Fusion in the Ascus.

Fig. 9. The fusing nuclei in the ascus. × 4,000.

Fig. 10. Sheaf-arrangement of the two chromatin masses, each mass attached to a 'central body'; lateral approximation of the chromatin masses. × 4,000.

Fig. 11. Fusion of the two nucleoli. × 4,000.

Fig. 12. Chromatin arranged in parallel strands. × 4,000.

Fig. 13. The resting condition of the fusion nucleus, showing chromatin sheaf attached to the small 'central body'. × 4,000.

Fig. 14. Synapsis in fusion nucleus. × 4,000.

Fig. 15. Stage resembling a spireme. × 4,000.

Figs. 16, 17. The five bivalent chromosomes; diakinesis. × 4,000.

The First Division in the Ascus.

Fig. 18. The intranuclear spindle; two centrosomes; metaphase of first division. × 4,000.

Fig. 19. Telophase. × 4,000.

Fig. 20. Reorganization of the two daughter nuclei. × 4,000.

Fig. 21. Two resting nuclei.

The Second Division in the Ascus.

Fig. 22. Metaphase of second division; centrosomes at poles.   × 4,000.

Fig. 23. Telophase.   × 4,000.

Fig. 24. Reorganization of the four daughter nuclei.   × 4,000.

The Third Division in the Ascus.

Fig. 25. Metaphase of third division; centrosomes at poles.   × 4,000.

Fig. 26. Telophase.   × 4,000.

The Formation of Ascospores.

Figs. 27, 27a. Centrosomes with radiating astral rays which are in contact with chromatin masses.   × 2,200.

Fig. 28. Cleavage of the cytoplasm delimiting the ascospores; the centrosomes and elongating chromatin.   × 2,200.

Fig. 29. Further cleavage; beaded chromatin threads.   × 2,200.

Fig. 30. Almost mature ascus and its eight ascospores; shortened chromatin threads; thickened cap at apex of ascus.   × 2,200.

Fig. 31. Transverse section of a ripe ascus showing arrangement of spores with sheaths. × 4,000.

JONES—RHYTISMA.

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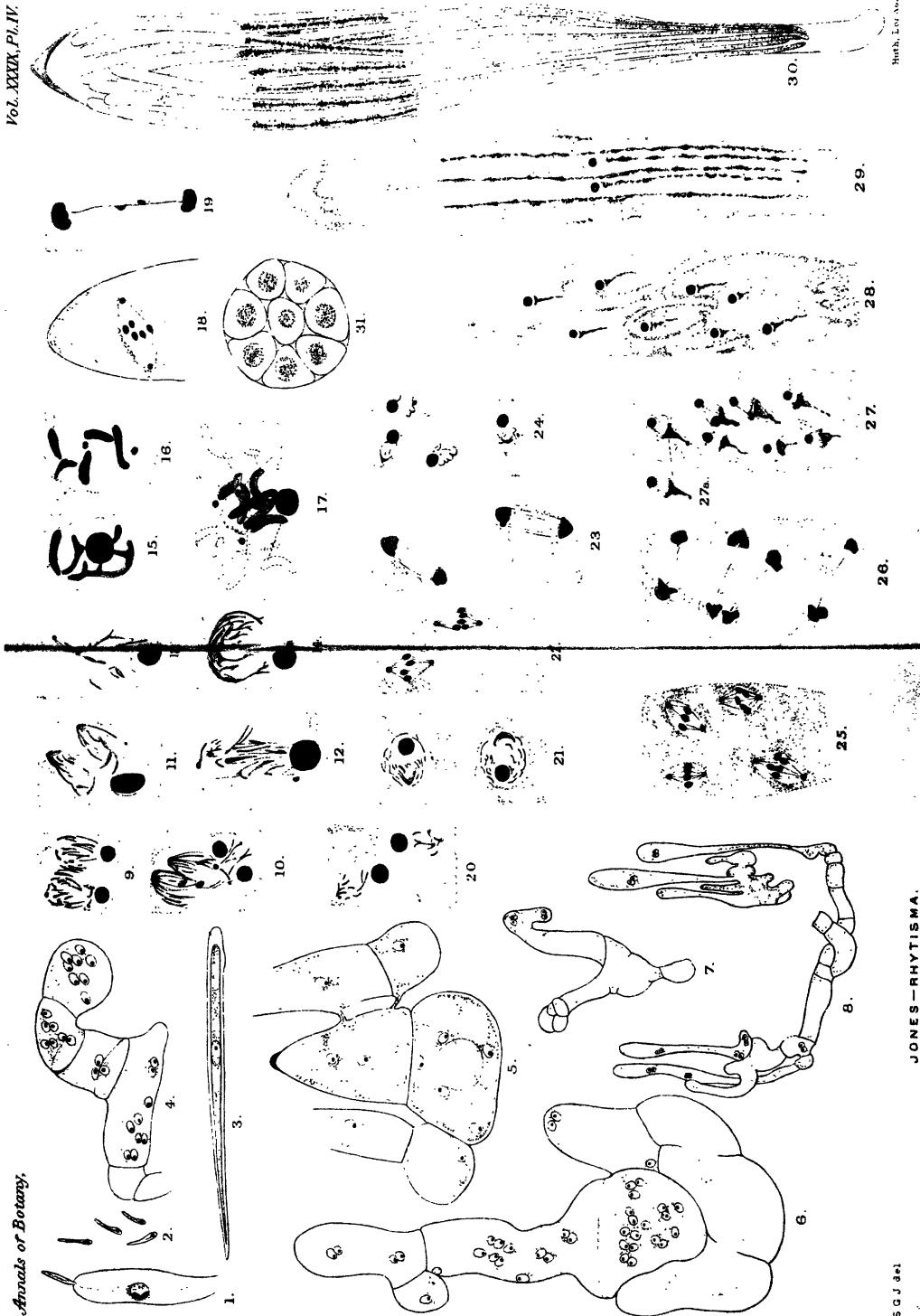
Chemical Studies in the Physiology of Apples.[1]

I. Change in the Acid Content of Stored Apples and its Physiological Significance.

BY

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With eight Figures in the Text.

APPLES of different kinds contain very different quantities of acid. At one end of the scale we find Sweet Alford, which, when ripe, has usually less than o·15 per cent. of acid, calculated as malic acid; at the other end such an apple as Bramley's Seedling, in which, when first picked, the acid may amount to as much as 1·5 per cent. Nevertheless, in spite of these large initial differences, the acidity of such apples drifts down to much about the same level if they are stored long enough; the juice of a really old apple of any variety has seldom an acidity much above N/50.

These changes of acid content, with their accompanying changes of hydrogen-ion concentration, can hardly fail to affect metabolism. The acid present in an apple cannot be entirely cut off from the protoplasm, for it is continuously drawn upon to provide material for respiration, and it is difficult to form any mental picture of this process of acid respiration which does not involve the assumption that the acid reaction of the proto-plasm is conditioned by the acid content of the vacuole. Moreover, it has been found experimentally that the rate of change of acidity is an index of the progress of those processes of break-down which may be grouped together under the head of senescence.

The present paper gives an account of investigations of changes in acidity in stored apples, which have been carried out through several successive seasons. A parallel investigation of hydrogen-ion concentration

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[1] It is proposed to include under this general heading a description of some correlated chemical and physiological investigations which are being carried out in connexion with researches on cold storage problems.

has also been undertaken, and will be described later. A further investigation of acidity in relation to other properties of the apple is in progress, and is being carried out in collaboration with Miss Helen Archbold and Miss Janet Brown.

<div align="center">EXPERIMENTAL.</div>

The expressed juice has in most cases been titrated with N/10 sodium hydroxide, using phenolphthalein as indicator. If the juice is largely diluted, and a considerable quantity of phenolphthalein is added, the end-point of the titration can be determined with sufficient accuracy. Titrations usually agree within 0·1 c.c., but the colour of the juice, and the buffering of the acid which it contains, militate against a sharp end-point, and these factors vary not only in different apples, but also in the same apples at different stages of their life. The error due to indeterminate end-point is, however, very small. A sharper end-point can be obtained by the use of potassium iodide and iodate, and this method gives very satisfactory results if the temperature is not allowed to rise above 20° C. Its use was discontinued in the present case to allow of titration after the addition of thymol, which is the most satisfactory preservative for liquids in which hydrogen-ion concentrations are to be determined.

Unless otherwise stated, ten apples were used for each determination. These were cut up, frozen, and, after thawing, pressed in a small hand-press, a thin cloth being used to contain the pulp. To ensure satisfactory freezing, the apple, cut into small pieces, was rammed down into a metal cylinder, immersed in a freezing mixture of ice and salt, and left thus overnight in a refrigerator kept at a low temperature. The juice was heated for an hour at 70°–75° C., immediately after it had been pressed out, to destroy pectinase and other enzymes.

It has been previously shown (Haynes and Judd[1]) that the juice obtained by fractional pressings of apple treated in this way showed no significant differences of titration, and this was taken to indicate that acid was not held back to any considerable extent in the pulp. The capacity of the press-residue to retain acid has now been further investigated ; additional series of fractional pressings have been carried out on apples of various kinds and at different stages of maturity, and a comparison has been made at intervals during the period of storage of the amount of acid obtained by alcoholic extraction of a weighed quantity of apple with that calculated from the titration of the juice for the same quantity. The results of these experiments are given below in Tables I and III. Table II illustrates the degree of uniformity obtainable by these methods. It gives the titrations

[1] Biochem. Journ., vol. xiii, p. 272, 1919.

of samples of juice from apples which had been cut up in small pieces, thoroughly mixed and frozen, and then pressed out in small separate lots.

TABLE I.

Acidity of Apple Juice expressed in Two Fractions.

Apple.	Fraction.	Volume of Fraction. c.c.	Titration mg. equiv. per 100 c.c.	pH.
Previous to storage (Sept. 10).				
Bramley's Seedling (rather	1	14	23.7	
immature)	2	12	23.45	
	1	27.5	23.9	
	2	15	23.6	
Ordinary store (Dec. 19–22)				
Dymock Red	1	90	3.85	4.11
	2	50	3.7	4.13
Allington Pippin	1	150	9.7	3.31
	2	30	9.55	3.33
Sweet Alford	1	130	1.35	4.48
	2	50	1.3	4.48
Bramley's Seedling	1	144	12.75	3.01
	2	52	12.85	2.99
Cold store (Dec. 19–22).				
Dymock Red	1	105	3.8	4.07
	2	36	4.15	4.03
Allington Pippin	1	110	13.65	3.14
	2	36	14.0	3.10
Sweet Alford	1	164	1.65	4.24
	2	35	1.75	4.24
Bramley's Seedling	1	165	13.75	2.99
	2	36	13.8	2.97

TABLE II.

Pressing Tests. Acidity of Samples mixed as uniformly as possible.

I. *Bramley's Seedling* (rather immature) September 10.

Sample.	Titration (mg. equiv. per 100 c.c.).
1	23.45
2	23.55
3	23.7
4	23.35

II. *Bramley's Seedling* (eight months in cold store) July.

1	8.95
2	9.3
3	8.75

III. *Worcester Pearmain* (ripe) September 30.

1	8.65
2	8.55
3	8.55
4	8.9
5	9.1
6	8.7

TABLE III.

Acid (calculated as Malic Acid) in 100 grm. of Fresh Apples (Bramley's Seedling).

From Canterbury.	*a.* *Estimated from* *Titration of Juice.*	*b.* *Estimated from* *Titration of Extract.*	*b.–a.*
Very immature, picked July 15.	2·498	2·380	−0·118
Immature, picked September 1.	1·475	1·475	0·000
From Spalding.			
Kept in cold storage at 1° C. until			
Nov. 29	1·105	1·068	−0·037
Dec. 12	1·103	1·113	+0·010
Jan. 24	1·086	1·096	+0·010
Mar. 19	0·957	0·972	+0·015
April 3	0·939	0·965	+0·026
„ 23	0·839	0·907	+0·004
May 15	0·800	0·808	+0·008
July 9	0·631	0·637	+0·006

The figures in column *a* are calculated from the formula

$$\frac{100 - K}{d} \cdot w,$$

where *K* is the residue left after alcoholic extraction of 100 grm. of fresh apple,

  d the specific gravity of the juice,

  w the weight of malic acid in 1 c.c. of juice.

The extracted acid was obtained as follows : 40 or 50 grm. of cut-up apple were weighed out from a well-mixed sample and frozen separately in a small cylinder. The juice was pressed out in the ordinary way, and the residue, after washing two or three times with alcohol, was extracted with alcohol overnight in a Soxhlet extractor, together with the pectin precipitated from the juice by the alcoholic washings. The press was washed with water and the pressing-cloth boiled out. After extraction the various acid-containing liquids—juice, alcoholic extract, and washings—were mixed, boiled to get rid of the greater part of the alcohol, and finally made up to a convenient volume and titrated in aliquot parts.

The results of Tables I and III show clearly that if there is any excess of acid in the liquid held by the press-residue this must be very small. Pressing in fractions makes little difference to the titration of the expressed juice, and the acidity as calculated from the titration agrees very closely in most cases with that determined directly from the extract. The young immature apples picked on July 15 are the only ones to show large differences, and this is very probably due to lack of uniformity in the samples ; for when acid is changing rapidly, as in this case, different apples

will differ largely in composition, and it is very difficult to obtain a uniform mixture. This is the more probable since the difference is negative, and all the stored apples except those of Nov. 29 show a small positive difference. This constant positive difference may indicate a very small hold-back, but if so, as has been observed, it must be very small. A large number of tests have been made for volatile acid in different samples of juice, and the amount has invariably been found to be negligible, though slightly greater in apples kept at ordinary temperatures than in those from cold store. In the immature apples there was no measurable quantity. It is, therefore, established that changes in titration of apple-juice may be taken as a measure of changes in the acid content of the apple throughout the period of storage.

✓The free acid present in apples is almost entirely organic. Its nature has not been investigated in the present instance, but in the light of Frauzen and Hebert's work on apple acids[1] it may be assumed provisionally that it consists essentially of a mixture of malic and citric acids in which malic acid largely predominates. Frauzen and Hebert give no indication of the kind of apple they examined, and their work was apparently carried out on a single batch of apples. This is unfortunate, for it would be extremely interesting to know whether apples of different kinds show differences in either the nature or the relative proportions of the acids they contain.

RATE OF LOSS OF ACID.

It has been already stated that the purpose of this paper is to describe the changes in total acid—measured by titration—which apples undergo in store. It will be convenient to begin this description with the study of a single set of apples, and to discuss their behaviour in some detail before attempting any general comparison. The apples in question were Bramley's Seedling, obtained in 1922 from an orchard in Spalding, Lincoln-shire. Bramley's Seedling is a variety which keeps well in store, and these apples showed quite exceptionally good qualities in this respect in both cold and ordinary store. They were stored at two temperatures, 15° C. and 1° C. The acidity at intervals during storage is shown in Table VI and graphically in Fig. 1.

A consideration of the figure makes clear that storage at low tempera-ture has two effects—(1) it decreases the rate of loss of acid, (2) it increases the fluctuations in the curve of acid content (cf. the two curves). Decrease in rate of respiration is, of course, a normal consequence of cold, but it will be shown in the sequel that this does not account for the whole effect in the case of acid, and that while apples in cold store are normally more acid than those kept at higher temperatures, this effect is dependent upon

[1] Zeitsch. f. Physiol. Chem., vol. cxxvii, p. 14, 1923.

the condition of the apple. The increase in the fluctuations of the acidity level is a somewhat surprising effect of cold storage. Fluctuations are, of course, a consequence of difference in individual apples in small
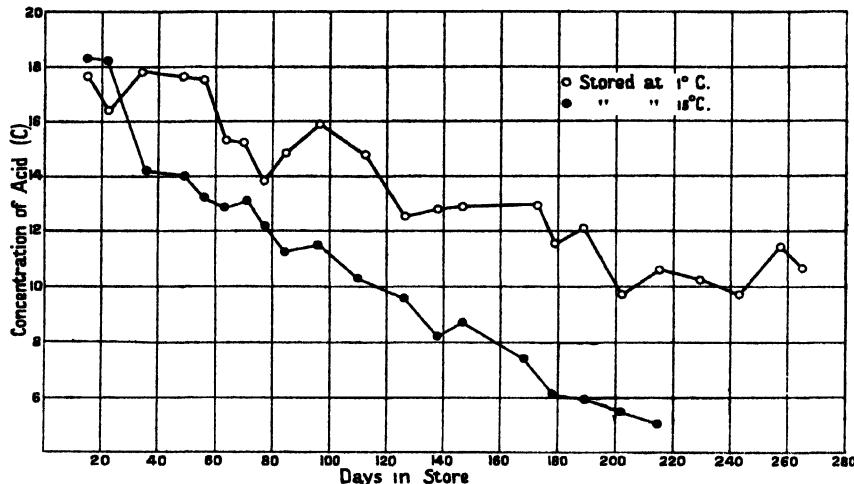
FIG. 1. Acidity of stored apples. Bramley's Seedling from Spalding, 1922–3.

samples; therefore, since cold storage tends to increase fluctuations, it must also increase divergence from the mean. That this is actually the case is shown by the results of Table IV, which gives examples of the acidity of sets of individual apples taken from store at the same time—two from the 15° C. store and one from that kept at 1° C. It will be observed that in

· TABLE IV.

Acidity of Individual Apples (Bramley's Seedling from Spalding, 1922–3).

C., concentration of acid (mg. equivalents per 100 c.c.).

	I.		*II.*		*III.*	
	From 15° C. store, Dec. 14.		*From 15° C. store, Feb. 27.*		*From 10° C. store, May 1.*	
	C.	*Deviation from Mean.*	*C.*	*Deviation from Mean.*	*C.*	*Deviation from Mean.*
1	15·35	+ 1·13	9·05	− 1·21	8·75	− 4·28
2	13·0	− 1·22	10·05	− 0·21	10·6	− 2·43
3	13·75	− 0·47	10·7	+ 0·44	13·9	+ 0·87
4	13·45	− 0·77	9·55	− 0·71	12·45	− 0·58
5	13·2	− 1·02	9·6	− 0·66	15·6	+ 2·57
6	14·0	− 0·22	11·4	+ 1·14	14·6	+ 1·57
7	13·5	− 0·72	9·5	− 0·76	14·15	+ 1·12
8	14·25	+ 0·03	10·35	+ 0·09	14·15	+ 1·12
9	16·4	+ 2·18	11·05	+ 0·79	10·6	− 2·43
10	15·3	+ 1·08	11·35	+ 1·09	15·5	+ 2·47
Mean	14·22	0·884	10·26	0·710	13·03	1·944
Per cent. of mean	—	6·2	—	6·9	—	14·9

apples from the 15° C. store the average deviation from the mean is under 7 per cent., while in those from cold store it amounts to very nearly 15

per cent. ; it would seem that those apples which draw most rapidly upon the supply of acid stored in the vacuole are those in which this loss suffers the least check in cold store. There are some indications that this be- haviour is related to the structure of the cell, and it may be for this reason that some apples, not only of different kind, but also of different origin, show it to a far greater degree than others. Table V gives the acidity of sets of apples (Lane's Prince Albert) from pruned and unpruned trees from similar plots at East Malling ; the specific gravity of the juice is also given, and it will be noticed that the mean deviation of both properties is greater in apples from the unpruned trees ; the number of observations is, however, too small to be conclusive, and the tables are given mainly as an illustration of the wide range of variation which may sometimes be en- countered. Late picking may also increase deviation from the mean, at least in respect of acidity. Figs. 4 and 5 are evidence of this. It should be remarked that the probable error of the mean acidity and the limits of significant difference have not been calculated from either set of results, since there is some doubt as to whether deviations from the mean conform to the frequency law.

TABLE V.

Acidity and Specific Gravity of the Juice of Individual Apples (Lane's Prince Albert from East Malling, 1922, taken from store at room temperature, Dec. 1922).

C., concentration of acid (mg. equivalents per 100 c.c.).
S., specific gravity.

	C.	Deviation from Mean.	S.	Deviation from Mean.
		Pruned Apple-trees.		
1	11·7	+ 0·68	1·048	+ 0·002
2	11·3	+ 0·28	1·040	− 0·006
3	11·6	+ 0·58	1·044	− 0·002
4	6·4	− 4·62	1·048	+ 0·002
5	12·15	+ 1·13	1·047	+ 0·001
6	8·25	− 2·77	1·051	+ 0·005
7	15·2	+ 4·18	1·042	− 0·004
8	11·55	+ 0·43	1·047	+ 0·001
9	10·95	− 0·07	1·042	− 0·004
10	11·15	+ 0·13	1·054	+ 0·008
Mean	11·02	1·49	1·046	0·003
Per cent. of mean	—	13·5	—	0·29
		Unpruned Apple-trees		
1	5·7	− 4·21	1·044	− 0·007
2	11·15	+ 1·24	1·054	+ 0·003
3	11·65	+ 1·74	1·058	+ 0·007
4	13·85	+ 3·94	1·052	+ 0·001
5	12·5	+ 2·59	1·069	+ 0·018
6	9·7	− 0·21	1·047	− 0·004
7	7·1	− 2·81	1·040	− 0·011
8	9·2	− 0·71	1·047	− 0·004
9	8·7	− 1·21	1·049	− 0·002
10	9·6	− 0·31	1·054	+ 0·003
Mean	9·91	1·90	1·051	0·006
Per cent. of mean	—	19·1	—	0·57

RATE OF LOSS OF ACIDITY.

A careful inspection of Fig. 1 shows that rate of loss of acid is not uniform, although through the greater part of the storage period the change of slope of the concentration-time curve is very small. If, however, the logarithms of the concentrations are plotted against time, a straight
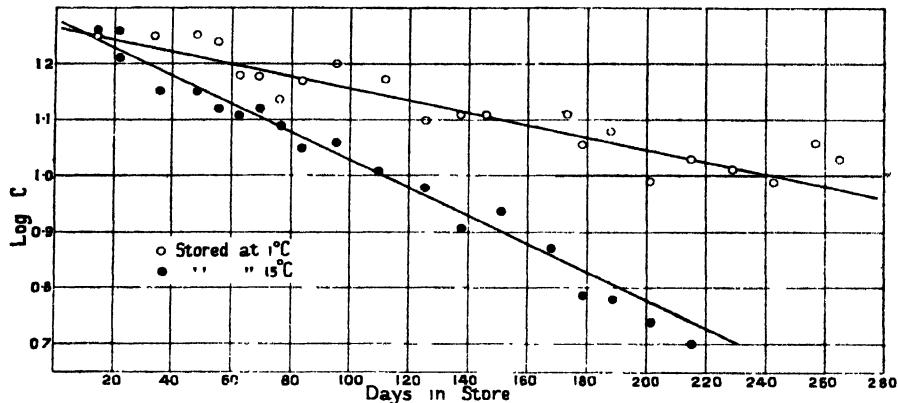
FIG. 2.   Logarithmic decline of acidity.   Bramley's Seedling from Spalding, 1922–3 (cf. Table IX, Nos. 1 and 2).

line can be drawn about which the points obtained are so evenly distributed (Fig. 2) that it seems justifiable to assume that the logarithmic relation is not merely a convenient empirical formula, but that it is the result of chemical reactions proceeding at definite rates, and can therefore be used
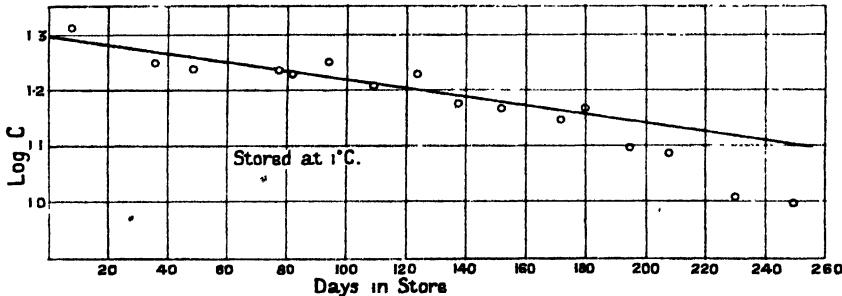
FIG. 3.   Logarithmic decline of acidity.   Bramley's Seedling from Spalding, 1923-4 (cf. Table IX, No. 3).

to draw conclusions as to these changes.   The rate of loss of acid in other sets of apples must, however, be first considered.   Table VI gives the concentration of acid in the juice of the above, and of other sets of Bramley's Seedling apples at intervals during storage ; the logarithms of the

TABLE VI.

Acidity of Stored Apples—Bramley's Seedling.

I. *From Spalding, Lincolnshire* (*Silt Soil*),
1922-3

	1° C. Store.		15° C. Store.	
Days in Store.	*C.*	*Log C.*	*C.*	*Log C.*
15	17·7	1·25	18·3	1·26
22	16·4	1·21	18.2	1·26
34	17.8	1·25	—	—
36	—	—	14·2	1·15
49	17·6	1·25	14·0	1·15
56	17·5	1·24	13·2	1·12
63	15·3	1.18	12·9	1·11
70	15·2	1·18	13·1	1·12
77	13·8	1·14	12·2	1·09
84	14·8	1·17	11·3	1·05
96	15·9	1·20	11·5	1·06
110	—	—	10·3	1·01
112	14·8	1·17	—	—
126	12·5	1·10	9·6	0·98
138	12·8	1·11	8.2	0·91
146	12·9	1·11	8·7	0·94
168	—	—	7·4	0·87
173	13·0	1·11	—	—
179	11·5	1·06	6·2	0·79
189	12·1	1·08	6·0	0·78
201	9·7	0·99	5·5	0·74
215	10·6	1·03	5·0	0·70
229	10·2	1·01		
243	9·7	0·99		
257	11·4	1·06		
265	10·6	1·03		

II. 1923-4.

	1° C. Store.	
Days in Store.	*C.*	*Log C.*
8	20·65	1·31
36	17·6	1·25
49	17·5	1·24
77	17·5	1·24
82	16.8	1·23
94	17·75	1·25
109	16·15	1·21
123	16·85	1·23
137	15·25	1·18
152	14·95	1·17
172	14·2	1·15
180	14·8	1·17
195	12·7	1·10
208	11·95	1·08
230	10·2	1·01
250	10·0	1·00

III. *From Burwell, Cambridge (Fen Soil),*
1922–3.

	1° C. Store.		15° C. Store	
Days in Store.	C.	Log C.	C.	Log C.
	(a) First picking, Oct. 10.			
14	19·2	1·28	18·8	·27
27	18·5	1·27	16·5	·22
40	19·0	1·28	16·3	·21
47	18·6	1·27	15·1	·18
54	17·7	1·25	13·8	·14
61	17·3	1·24	13·4	·13
76	15·7	1·20	12·5	·10
89	16·8	1·22	12·1	·08
96	15·9	1·20	11·9	·08
110	15·7	1·20	10·2	·01
119	14·1	1·15	8·9	0·95
126	14·1	1·15	8·8	0·94
140	14·1	1·15	7·5	0·88
152	11·3	1·05	6·4	0·81
159	13·5	1·13		
166	12·3	1·09		
	(b) Second picking, Nov. 6.			
	16·4	1·21	14·9	·17
15	15·3	1·18	12·6	·10
22	14·9	1·17	13·7	·14
34	15·7	1·20	13·7	·14
43	—	—	11·4	·06
49	14·2	1·15	—	—
57	13·6	1·13	12·1	·08
64	15·0	1·18	11·4	·06
71	12·1	1·08	10·2	·01
78	11·7	1·07	8·1	0·91
85	15·0	1·18	9·2	0·96
97	13·8	1·14	8·5	0·93
104	—	—	7·5	0·88
107	11·5	1·06	—	—
118	12·1	1·08	7·2	0·86
127	14·6	1·16	7·4	0·87
139	10·5	1·02	6·0	0·78
147	8·4	0·92	6·3	0·80
169	7·0	0·85	5·2	0·72
180	6·3	0·80	5·0	0·70
190	10·0	1·00	4·1	0·61
202	4·3	0·63	4·3	0·63
216	6·7	0·83	3·9	0·59
230	4·3	0·63		
258	4·4	0·64		

concentrations are also given, and these are plotted against time in Figs. 2–5. In order to show that this behaviour is general and not specially character-istic of Bramley's Seedling apples, a similar graph is given of observations shown in Table VII made on Cox's Orange Pippin; the low temperature curve for these apples shows the break-away very markedly indeed. It will be noticed that there is usually an increase in rate of loss of acid towards the end of the season. This increase is the concomitant of physiological break-down; it normally sets in when acid has become low and the cell-wall has undergone a considerable amount of disintegration,

but in some sets of cold-stored apples a premature break-down occurs while the cell-wall is still intact and the acid content high : in this case there is a rapid falling away from the normal direction of the concentration-time curve. The condition is well exemplified in Figs. 3 and 5, in which a sharp change of direction is to be observed in the 1° C. graphs.

TABLE VII.

The Acidity of Stored Apples—Cox's Orange Pippin, 1922–3.

Days in Store.	1° C. Store.		15° C. Store.	
	C.	Log C.	C.	Log C.
	Oct. 1.			
14	11·1	1·05	8·2	0·91
23	11·2	1·05	7·6	0·88
42	10·3	1·01	7·1	0·85
56	9·6	0·98	5·4	0·73
70	8·7	0·94	4·8	0·68
93	8·0	0·90	3·9	0·59
101	6·2	0·79	3·6	0·56
115	4·3	0·63	3·4	0·53
117	4·1	0·61	—	—
124	—	—	2·2	0·34
129	3·4	0·53		
143	2·2	0·34		
148	3·5	0·54		
170	2·3	0·36		
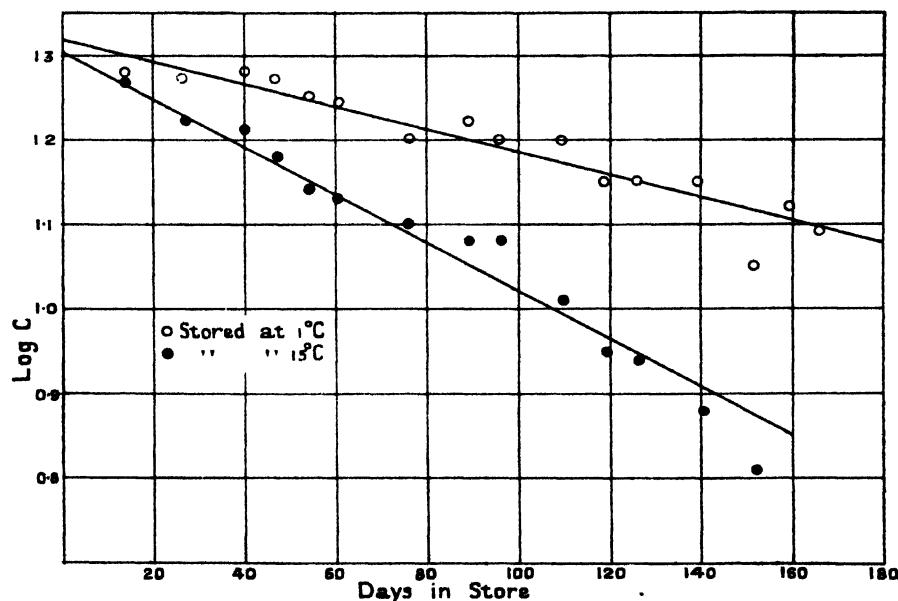
FIG. 4. Logarithmic decline of acidity. Bramley's Seedling from Burwell, 1922–3. First picking (cf. Table IX, Nos. 4 and 5).
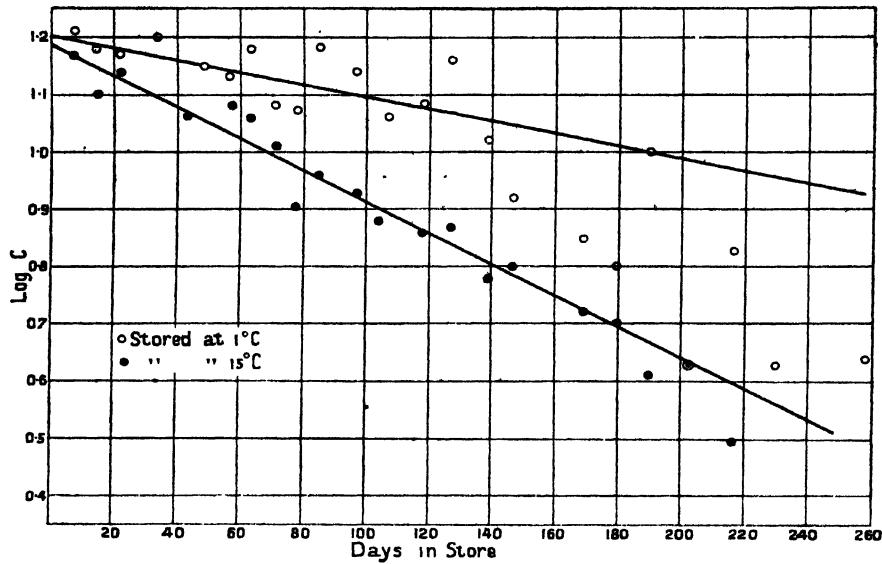
FIG. 5. Logarithmic decline of acidity. Bramley's Seedling from Burwell, 1922–3. Second picking (cf. Table IX, Nos. 6 and 7).
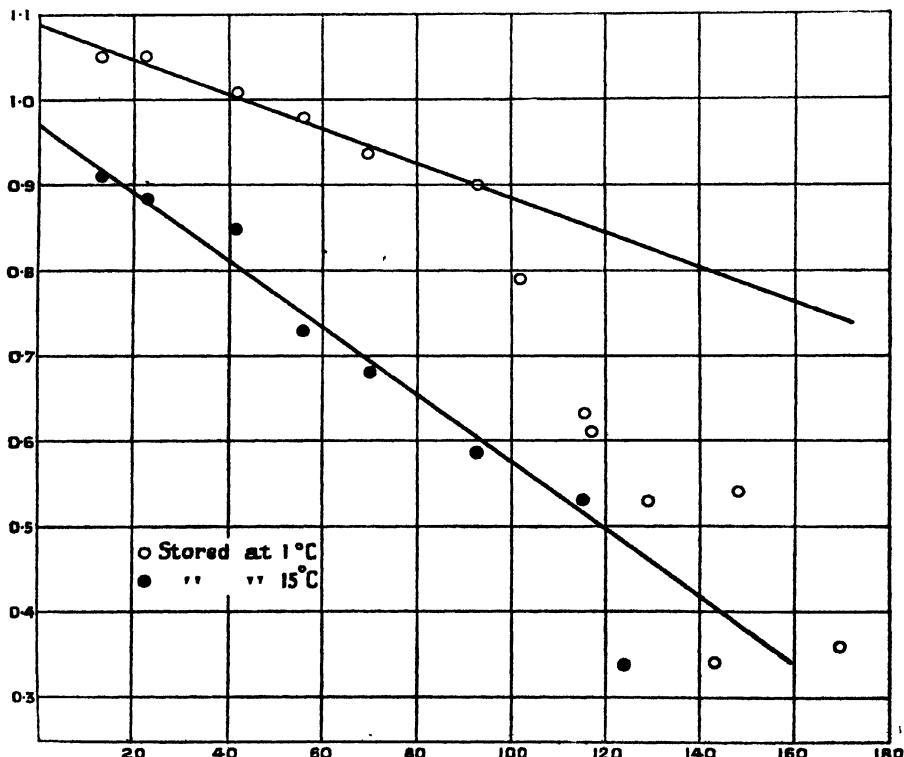
FIG. 6. Logarithmic decline of acidity. Cox's Orange Pippin, 1922–3 (cf. Table IX, Nos. 8 and 9).

In order to obtain more definite information the constants of the equation

$$\log C = b - at$$

have been calculated by the method of least squares from the various sets of observations. The lines thus obtained are drawn through the points plotted in Figs. 2–7. The earlier observations alone are used for this purpose, since later in the season incipient physiological break-down is likely to affect the slope of the line, but this occurs very early in certain cases and the calculated slope is then too steep. The values of the constants, the range of observations from which they are calculated, and the number of days of storage before the last observation are shown in Table VIII.

TABLE VIII.

Constants of the Equation $\log C = b - at$.

	Temp. of Store.	No. of Observations.	Storage Period.	Constants. $a \times 10^5$.	b
Bramley's Seedling.					
1 Spalding, 1922–3	1° C	17	189 days	108	1·264
2	15°	14	146 ,,	247	1·278
3 ,, 1923–4	1°	12	180 ,,	78	1·298
Burwell, 1922–3.					
4 First picking	1°	16	166 ,,	133	1·320
5	15°	14	152 .,	286	1·307
6 Second picking	1°	15	139 ,,	98	1·204
7	15°	16	147 ,.	277	1·194
Cox's Orange Pippin.					
8 1922 3	1°	6	93 ,,	203	1·089
9	15°	8	115 ,,	404	0·976

The graphs given in Figs. 2–6 show how the points obtained by observation are scattered about the straight lines drawn according to the calculated constants. The lines lie very close to the mean position of the points in every case until the break in the curve occurs. when, as always, they begin to scatter (cf. especially the 1° C. graphs in Figs. 5 and 6).

The constant *b* in the above equation represents the value of the logarithm of the titration at the time the apples were picked. Since acidity is always on the down grade at the time of picking, this value is somewhat arbitrary, but it should be the same for all temperatures of storage. When this is not the case deviations from the logarithmic law must have occurred, and these will also be reflected in the value of *a*, which measures the rate at which the acidity declines. Deviations appear to arise from two principal causes: (1) *Immaturity*, as when apples are picked very

early. (2) *Internal break-down in cold store.* The physiological disturbance which produced this condition appears to proceed at very different rates in different cases; when it arises suddenly a sharp change of slope occurs in the logarithm curve (cf. Figs. 3, 5, and 6), but it may also proceed gradually, and the change of slope is in this case much less noticeable. The first picking of Burwell Bramleys (No. 4) behaved in this way; acid fell off slowly at first, and a change may be observed after about seventy days; the calculated line shown in the figure has there-fore a steeper slope than would be obtained from the first six or eight points. The corresponding incidence of internal break-down is shown in Table IX.

There appears to be a very direct association between high acidity and internal break-down in cold store, and there seems good reason to believe that internal break-down might be almost completely avoided if apples were not exposed to low temperatures until their acid content had been reduced. When they are picked with a high content of acid and their constitution is such that they tend to lose acid slowly, conditions appear to be most favourable to internal break-down. These conditions were fulfilled by the Spalding Bramleys in 1922–3, and the loss from internal break-down was very high.

It is noticeable that apples stored at 15° follow the logarithmic law more closely than those stored at 1° C., and that at this temperature there is little evidence of change of slope until the end of the season. This is no doubt because premature physiological break-down does not normally occur. It should be remarked that the true rate of loss of acid is higher than the observed rate, since water loss tends to concentrate acid in the juice, but since both processes take place at a rate proportional to existing concentra-tions they are both logarithmic. At 1° C. transpiration losses are almost negligible.

It has already been shown that cold storage tends to increase the natural variability of apples in respect of acid content. There is much reason to believe that rate of loss of acid is largely conditioned by the rate at which acid is produced in the apple by the oxidation of sugar, and that it is this production of acid which is differentially affected by low tempera-tures. The cause of this differential effect, which is intimately associated with liability to internal break-down, remains to be ascertained. It may be associated with the structure of the apple, with its content of sugars, salts, or other substances, or with other differences at present undefined. Investiga-tions are proceeding with the object of obtaining further information on the subject, which is undoubtedly of fundamental importance for the storage of fruits. Table IX shows that time of picking may be important in this connexion, probably on account of differences of acidity. In Figs. 7 and 8 the acidity of different pickings of Bramley's Seedling are directly compared,

the acidity being in this case plotted against dates instead of against times
of storage. These graphs show that there is a slight tendency for higher
acid to approximate to lower (also shown in Table VIII by decreasing value
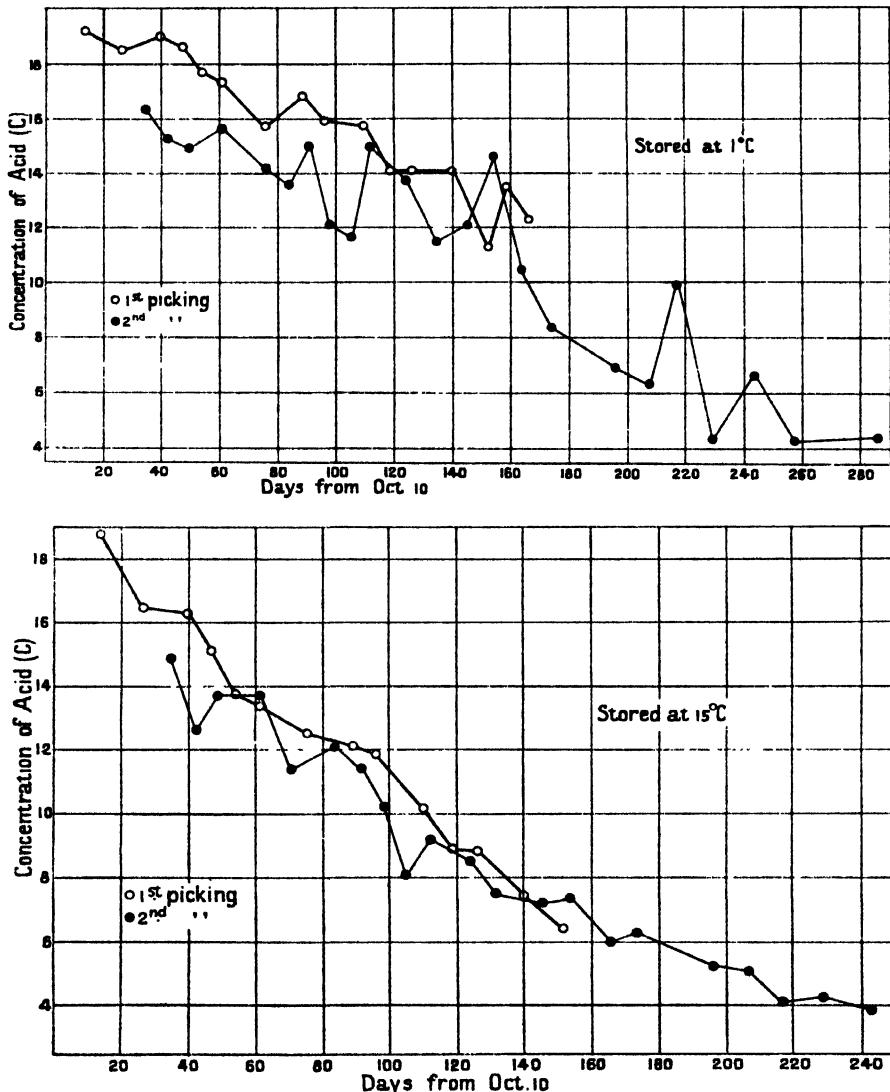
FIGS. 7 and 8. Acidity of apples picked at different dates. Bramley's Seedling from Burwell,
1922–3. Two pickings : (1) Oct. 10 ; (2) Nov. 6.

of *a*), but that the rate of approximation is very slow. Similar behaviour
was shown by Lane's Prince Albert apples when picked at intervals of some
weeks.

TABLE IX.

Internal Break-down at 1° C. (*Data from Dr. C. West.*)

Apple.		Percentage of Break-down.		
Bramley's Seedling—	Jan.	Feb.	March.	April.
Spalding, 1922–3	2	2	20	55
Burwell, 1922–3—				
First picking	4	80	100	—
Second „	0	47	80	95
Cox's Orange Pippin,				
1922–3	10	30	43	68
Bramley's Seedling—	Jan. and Feb.	March.	April and May.	June.
1923–4	0	0·5	55	88

GENERAL INTERPRETATION OF RESULTS.

It is clear from the foregoing that the logarithmic law is at least a close approximation to the rate of fall of concentration of acid in stored apples, i. e. the concentration falls at a rate proportional to itself, so that $-dc/dt = ac$. The interpretation of the results here described has been greatly facilitated by parallel measurements of rates of respiration which have been carried out by Dr. Franklin Kidd and Dr. Cyril West during the season 1923–4 at Cambridge on the Bramley's Seedling apples from Spalding stored at 1° C. Further investigations on these lines are in progress, and it is hoped to describe the joint experiments in detail elsewhere, but the writer is indebted to the above investigators for the following information. During the first 100 days in store the rate of respiration increased from 15·5 c.c. to about 26 c.c. per 10 kilo-hours; the average output of CO_2 may therefore be taken for purposes of rough calculation as 20 c.c. per 100 grm. per 100 hours, which is equivalent to a consumption of 0.64 grm. sugar or 0·72 grm. malic acid. The loss of acid during the same period—the first 100 days in store—can be calculated from Table VIII, and amounts to 1·2 mg. equivalents, i. e. 0·08 grm. malic acid per 100 c.c. of juice, which is equivalent to about 105 grm. of apple. The stored acid consumed represents, therefore, less than one-ninth of the total respired material. The break in the curve after May 1 indicates a marked acceleration in the rate of loss of acid. This was found to be accompanied by a decrease in the rate of output of CO_2.

There is much evidence that acid is produced during the process of sugar respiration; it may therefore be assumed that the difference between the rates of production and consumption of acid determines the rate of loss of the acid contained in the vacuole. As long as the logarithm law holds, this rate is also proportional to the concentration of acid; hence

$$- dc/dt = a_1 C = a_2(x-y)$$
$$\text{and} \quad x-y = A_1 C$$

where x and y are the rates of consumption and production of acid.

Under the same conditions the concentration of acid in the proto-plasm (Cp) may be taken as proportional to that in the vacuole p (taken as equivalent to the total concentration C); for, assuming that the protoplasm is the seat of active chemical change, there must be a steady drift from vacuole to protoplasm, the rate of which should be proportional to the gradient $C-Cp$; hence

$$-dc/dt = a_3\,(C-Cp)$$
$$= a_1,\,C \text{ (from result above)}$$

therefore $\qquad\qquad Cp = A_2 C.$

Thus if, as is probable, the rate of consumption of acid is proportional to its concentration in the protoplasm, the rate of production of acid must also be proportional to the same concentration while the logarithmic law holds.

Little or nothing is known about the mechanism of acid production in the living cell, but it is very probable that it is an integral part of the process of respiration. The results discussed above suggest that during the storage life of the apple acid production keeps pace with acid concentration and is proportional to it. It is, however, impossible to decide whether this relation is exact or only approximate; the observed points indicate small changes of slope, but such changes may often be merely the result of a large sampling error. It may be simply that the rate of change of sugar to acid by oxidation is running down at approximately the same rate as the concentration of acid.

It is hoped to obtain further light upon respiration processes, by a more detailed study of the conditions which determine physiological break-down, the beginnings of which are indicated by a break in the logarithmic curve. It has already been pointed out that physiological break-down in the cold is probably associated with conditions which keep up acidity; a high rate of production of acid, a high value of Cp, and a low value of the gradient $C-Cp$ would, therefore, favour internal break-down.

It may be that a special sugar is the precursor of acid, and that the supply is drawn upon more rapidly under special physiological conditions. It is proposed to investigate the rate of inversion of cane sugar in this connexion, since the concentration of cane sugar decreases rapidly in stored apples, and it is possible that cane sugar may derive a special importance from its active fructose group. Other sugars, e. g. glucose, may also be used up, but it is difficult to understand a sudden break-down on this account. The observations suggest that, if the phenomenon of physiological break-down is to be explained by the disappearance of some substance essential for the production of acid, the substance must at first be present in excess, so that the active mass of enzyme is the determining factor; decline would then set in from the time that the mass of this substance began itself to determine the rate of reaction. Other explanations on similar lines are

also possible, e. g. a sudden inhibition of enzyme activity such as might occur from an accumulation of metabolic products.

It should be pointed out, however, that explanations of an entirely different character are also not excluded. Protoplasm is generally believed to exist as a colloidal sol whose state of aggregation is labile and largely determined by the concentration of the electrolytes and perhaps also of the sugars it contains. The degree of dispersion probably lessens with age as in ordinary colloidal solutions, and it is not improbable that coagulation sets in when the concentration of sugars and acids which exert a peptizing action becomes low. This process would probably take place more rapidly in the cold, since the coagulation of emulsoid colloids appears to be accelerated by low temperatures; physiological break-down in cold store would thus be explained, and the increased consumption of stored acid which characterizes the initial stages of break-down might be due to increased permeability inducing a more rapid diffusion from the vacuole. Other physical changes might also be suggested as initiating a general disturbance of metabolism, but a discussion of the balance of evidence in favour of a physical or chemical interpretation of the phenomena of physiological break-down is premature until further investigation has been carried out upon the chemical side.

THE RELATION OF CHANGE OF ACIDITY TO SENESCENCE.

It has been shown that while the rates at which apples lose acid is variable, it is always logarithmic. Disintegration changes in the cell-wall appear to keep pace with acid changes, so that apples which 'keep' well are those which lose acid slowly. The changes which result in physiological break-down appear to be of a different character, and evidence has been given that they are accelerated by low temperatures. Conditions which favour the maintenance of a high acid level in cold store appear to increase susceptibility to physiological break-down while they retard the break-down of the cell-wall, and thus the condition of premature, i.e. 'internal', break-down in cold store is probably brought about.

RATE OF CHANGE OF ACIDITY AS AN INDEX TO STORAGE LIFE.

The rate of progress of disintegration in the cell-wall is probably determined by hydrogen-ion concentration; for this reason the titration can be used as an approximate index of conditions for all apples of the same kind. It should, therefore, be possible to forecast the life of a batch of stored apples by titrating the juice of representative samples at two or three different periods in the first weeks of storage; the logarithmic curve thus obtained would show the time required for the acidity to fall to any

given concentration. The lowest concentration compatible with a satisfactory condition of the apple would of course vary with different kinds; for Bramley's Seedling 10 mg. equivalents per 100 c.c. would probably represent the limit. The same observations would indicate that liability to premature break-down in cold store should be suspected whenever an abnormally small slope of the logarithmic curve occurred in conjunction with high acid. In order to ascertain the practical value of this method a number of estimations of rates of loss of acidity are being carried out on apples submitted to special storage tests at Cambridge.

SUMMARY.

It has been shown by fractional pressing, and also by exhaustive alcoholic extraction, that the acid content of apples can be determined within narrow limits by the titration of their juice.

The effect of storage at low temperature is (1) to decrease the rate of loss of acid, (2) to increase the fluctuations of acid content due to differences of sample.

Tables are given showing variations in the acidity of individual apples at ordinary temperatures and in the cold.

The rate of loss of acid is shown to follow a logarithmic law, and the constants of the equation

$$\log C = b - at$$

are calculated for various sets of apples. Graphs are given showing the distribution of points about these lines.

Departures from the logarithmic law are usually correlated with the incidence of internal break-down, the first apparent effect of the onset of this condition being a more rapid rate of loss of acid. A table is given for comparison with the above-mentioned graphs (from information supplied by Dr. C. West), showing the incidence of internal break-down in the same apples.

These observations indicate that high acidity and a slow rate of loss of acid are conditions favouring internal break-down, and that this would probably be greatly lessened if apples were not exposed to low temperatures until their acid content was reduced.

It is probable that rate of loss of acid in the apple is largely determined by the rate at which sugar is oxidized, and that it is the rate of production of acid which is differently affected in different apples. The cause of this differential effect, which is intimately associated with susceptibility to internal break-down, is under investigation.

The implications of the logarithmic law are discussed, and the rate of loss of acidity in a set of Bramley's Seedling apples is compared with their rate of respiration.

A method for forecasting the storage life of apples is suggested.

In this, the first paper of the series, the writer desires to express on behalf of herself and her colleagues their great indebtedness to Professor V. H. Blackman for his continuous interest in the work and for much help in its development. They also wish to thank Dr. Franklin Kidd and Dr. Cyril West for arranging for the supply of apples and for the facilities accorded them at the Low Temperature Research Station at Cambridge. Most of the apples investigated have been stored at Cambridge, and the very accurate control of temperature and other conditions maintained there has been of first importance for the success of the work.

Chemical Studies in the Physiology of Apples.

II. The Nitrogen Content of Stored Apples.

BY

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With two Figures in the Text.

THIS work has been undertaken in an attempt to determine the possible significance of nitrogen in the metabolism of the apple and in the changes leading to break-down of the fruit. This has involved (1) a preliminary survey of the total amount of nitrogenous substance present in different varieties of apple and in the same variety grown in different localities ; (2) determination of the sampling error, and its variation throughout the storage life ; (3) investigation of the nature of the nitrogenous material present ; (4) observation of the changes of total nitrogen during storage ; and (5) correlation of these data relating to nitrogen with changes in other constituents.

To obtain results which can justifiably be compared the choice of a standard of reference for a fruit which in storage is continuously losing weight is of first importance. For this reason, estimations of total dry weight and water-insoluble material were carried out concurrently with the nitrogen estimations. The determination of ash content, which must be invariable once the fruit is gathered, was impracticable owing to the large amount of material required and the labour involved. The water-insoluble material was therefore originally chosen as the least variable constituent, and also for its possible physiological significance as a measure of the amount of cell-wall material present.

As the work progressed, however, it appeared that the nature of the nitrogen curve of cold-stored apples (1° C.) was not much affected by the basis of reference. The hydrolysis of the cell-wall was more rapid than was anticipated, and the fresh weight, dry weight, and water-insoluble

material were found to maintain a constant ratio to one another. The influence of increase in temperature, however, was marked—the total loss of weight if the life is prolonged may at 3° C. be as much as 30 per cent. as compared with 10 per cent. at 1° C. The ratio of percentages of the constituents in this case varies with time, and in the absence of any physiological criterion of maturity the only reliable basis seems to be the fresh weight at time of storing; accordingly the data giving the nitrogen changes are not only referred to the weight of the apple at time of analysis, but are also calculated back to the original fresh weight. Unfortunately, data of the original fresh weights were not available for all samples. In such cases the original fresh weights have been calculated where possible from the known rate of loss over a given period, assuming the losses to be constant under the standard conditions of storage.

The total amount of nitrogen present is 0·02 to 0·06 per cent. of the fresh weight according to variety; individual apples from the same sample may vary as much as 40 per cent. Experiments described in the present paper show that the presence of other forms of nitrogen than protein is extremely doubtful. The nitrogenous substances seem in fact to be little more than cytoplasmic protein, and thus the total nitrogen content of given samples may be regarded as a comparative measure of the protoplasm present. In this research a correlation is observed between nitrogen content and rate of respiration, a high nitrogen value being associated with a high respiration rate; this indicates that the rate of oxidation in the apple is controlled by the amount of protoplasm present.

I. Determination of Total Nitrogen.

The Kjeldahl method of estimation was used. As shown in the sequel, a modification to include nitrates appeared unnecessary, as none could be detected in the sap. A large number of experiments were carried out to ascertain the best conditions of analysis. A standard apparatus was used with 400 c.c. digestion flasks and litre distilling flasks. The errors of manipulation were found by testing the apparatus with pure asparagine, and were found to be 0·2 c.c. in titration, using a quantity equivalent to 11 c.c. $N/10$ H_2SO_4,—i.e. approximately two per cent. The mixed pulp of apples cut finely by hand was used in 50-gramme lots. Although the amount of ammonia obtained is small, the large percentage of sugars present rendered the digestion of a larger sample difficult, and increasing the amount of carbohydrate to be oxidized in itself tends to increase the error. The fresh material was almost impossible to digest, and removal of most of the water was imperative. After comparison of results of partial drying at 30° C. in a current of air and complete dehydration at 100° C. the latter method was adopted. The results were not affected by the

different temperatures of drying, and the Kjeldahl process was greatly facilitated by using dry material. The 50-gramme lots were therefore dried for thirty-six hours on filter-papers, and transferred with the papers to Kjeldahl flasks ; 50 c.c. H_2SO_4, 10 grammes $KHSO_4$, and a crystal of copper sulphate were used for digestion, which was complete in three to four hours. The digestion as a rule proceeded quietly, frothing only occurring in a very few cases, but it was very necessary to rinse down the sides of the flasks after the initial evolution of SO_2 was complete. The clear liquid was washed out into litre flasks, made alkaline with soda, and distillation carried on into 10 c.c. of $N/10$ H_2SO_4, using methyl red as indicator.

Errors due to Inefficient Mixing.—The maximum difference of ten estimations of several types of apple varied from 4 to 12 per cent. referred to the fresh weight. This difference is due almost entirely to inefficient mixing, the use of more homogeneous material reducing it considerably ; improved mixing was obtained by grinding up the dried tissue. After drying for twenty-four hours the tissue could be readily ground in a glass mortar to a fine powder, which was very hygroscopic. Dried and weighed test-tubes ($1'' \times \frac{3}{8}''$) were filled with this material and placed in weighing bottles and again dried for six hours, and cooled in a desiccator over sulphuric acid. The maximum difference from the mean in this case did not exceed 3 per cent. The following are typical sets of results :

TABLE I.

Unground Material %N. (fresh weight).	Ground Material %N. (dry weight).
0·0209	0·399
0·0210	0·397
0·0225	0·407
0·0233	0·397
0·0210	0·389
0·0228	0·390
0·0218	0·388
0·0216	0·398
0·0224	0·399
0·0226	0·398
Mean 0·0220	Mean 0·396
Probable error of mean ± 0·0005 or 2·2%	Probable error of mean ± 0·003 or 0·8%

Efficient mixing of unground samples reduces the errors considerably. The large differences at first obtained were due to the cutting being too coarse and the sample being insufficiently mixed ; an agreement within five per cent., however, is the closest possible with an unground sample. In view of the large sampling error this is close enough for ordinary work. Blank determinations were made in all cases and the difference in titration subtracted.

Analyses of Different Varieties of Apple.—Using this method, the following results were obtained on analysis of various types of apples :—

TABLE II.

Variety.	% N (fresh wt.).	Bramley's Seedling from various sources.		
		Source.	Soil.	% N (fresh wt.).
Cox's Orange Pippin	0.038	Wisbech	Silt	0.0359
		Worcester	Old Red Sandstone	0.0362
Lane's Prince Albert	0.053	Canterbury	Gravel	0.0334
		Bristol	Sandy Loam	0.0408
Red Dymock	0.0423	Burwell	Fen	0.0420
		,,	Chalk	0.0351
Sweet Alford	0.0361	Spalding	Silt	0.0280
Allington Pippin	0.0325			

These estimations were carried out during November 1923, after the apples had been in store about six weeks. No allowance has been made for the possibility of the fruit being at different stages of senescence, but the chemical changes during the early period of storage are known to be small, so that the figures may be regarded as comparable. The object of the analyses was to give some idea of the order of difference to be expected both in different varieties and in the same variety under widely different conditions. Except for the high value for Lane's Prince Albert, the figures do not appear to be characteristic of the varieties, the Bramley's Seedling apples, according to their environment, covering the whole range of values.

II. SAMPLING ERRORS.

These have been determined by a number of estimations on sets of ten individual apples, using 40 grammes of pulp from each apple. The variety used was Bramley's Seedlings, and the fruit was stored at 1° C. and 3° C. The results are tabulated on p. 101.

The size of the probable errors is such that non-significant differences of 20 per cent. may occasionally be expected. Owing to the large amount of mechanical labour involved, it is not practicable to increase the number of apples employed in a sample to such an extent as markedly to reduce the size of the error. It is evident, therefore, that in the comparison of a series of results, occasional fluctuations of considerable size must be neglected ; in the absence of really large variations only the general trend of the results is significant.

TABLE III.

Nitrogen Content of Single Apples in Terms of Fresh Weight at Time of Analysis.

Date of Analysis.	Temperature of Storage 1° C.			Temperature of Storage 3° C.		
	Apr. 30	*May* 28.	*Aug.* 5.	*Mar.* 6.	*May* 19.	*Aug.* 12.
	0·0271	0·0208	0·0193	0·0290	0·0314	0·0287
	0·0257	0·0231	0·0270	0·0342	0·0248	0·0265
	0·0119	0·0238	0·0357	0·0290	0·0245	0·0315
	0·0209	0·0178	0·0196	0·0306	c.0273	—
	0·0227	0·0204	0·0286	0·0377	0·0263	0·0223
	0·0280	0·0265	0·0223	c.0244	0·0301	0·0253
	0·0174	0·0272	0·0181	0·0335	0·0334	0·0352
	0·0354	0·0212	0·0230	0·0503	0·0191	0·0282
	0·0179	0·0250	0·0255	—	0·0297	0·0255
	0·0278	0·0232	0·0241	0·0335	0·0273	0·0313
Mean	0·0235	0·0229	0·0241	0·0302	0·0274	0·0283
Maximum diff. from mean.	50 %	25 %	48 %	66 %	30 %	42 %
Probable error of mean of 10 results.	6·11 %	3·6 %	4·62 %	4·8 %	3·17 %	3·51 %

III. NATURE AND DISTRIBUTION OF NITROGENOUS SUBSTANCES.

Observation of the relation between soluble, colloidal, and insoluble forms of nitrogen has presented considerable mechanical obstacles, chiefly arising from the difficulty of making any adequate separation of the small quantities involved, and the impossibility of precipitating quantitatively small amounts of colloidal matter in the pulp extracts. The general method followed is adapted from that used by Chibnall (1, 2) for the extraction of proteins from leaves. The apple pulp, previously frozen, was pressed in a small hand-press through a fine-meshed cloth. The pressed residue was washed with distilled water and again pressed; the process being repeated (2–3 times) until no more nitrogen was found in the extract.

The first runnings from the press contain about twenty per cent. of the total N, and represent nearly all that can be extracted by water. By subsequent washing and pressing, no more N was found in the extract after 100 c.c. of water had passed through a 100-gramme sample. The possibility exists that minute quantities might be being expressed by further washing, either owing to gradual disintegration of the tissue by continued pressing or slight hydrolysis of insoluble protein. In order to test the completeness of extraction a sample was autoclaved after being pressed and washed as above. This process serves to separate the cells completely. On further pressing, however, sufficient nitrogen for estimation

was not extracted. Attempts to detect the commoner water-soluble nitrogenous substances in the extract produced negative results even with large quantities of juice. No evidence of the presence of nitrates or amino-acids was forthcoming, while the trace of ammonia which was found is only detectable by Nessler's reagent, and is probably due to the action of the magnesium oxide on the colloidal proteins, since it showed no variation in storage as time went on. These observations show that at most the crystalloidal N in solution is very small in quantity, and that the nitrogenous substances in the sap are nearly all colloidal. Microscopic examination of the extracts showed that as washing proceeded, fragments of the cell contents were expressed, although hardly any particles were visible in the first runnings. The later extracts were therefore filtered. The pressed tissue consisted of cells still retaining their protoplasmic contents, and only a few of which were torn. The first pressing, therefore, apparently expresses the vacuole fluid, together with colloidal material from the protoplasm of the cell. The washings remove the rest of the vacuole fluid held back in passing through the permeable walls, and, owing to the rupturing of some cells, more or less of the protoplasmic contents. It seems probable that. as in the case of leaves (Chibnall (2)), very little of the nitrogen is originally in the vacuole, most of that in the expressed sap coming from the protoplasm ; but his method has not been applied to the apple tissue.

The quantitative precipitation of the colloidal protein in the sap has so far not been possible. No coagulation occurred on heating, and addition of $CaCl_2$ produced a solution which it was impossible to filter. Both alcohol and phosphotungstic acid precipate 50–60 per cent. of the sap N, but the amount brought down seems to vary with the composition of the sap. The phosphotungstic acid gave the most consistent results, but these were always lower than those obtained with alcohol.

The remaining nitrogen in the tissue cannot be extracted by alcohol, and only a very small fraction is extracted by dilute HCl or NaOH. Increasing strength of acid removes a larger proportion, but even after removal of the sugars with alcohol and prolonged hydrolysis with 20 per cent. HCl over one-third of the total remained in the residue.

The variation in the nitrogen of the juice during the period of storage is shown below. These figures represent the difference between the nitrogen found in the extracted and unextracted pulp, the Kjeldahl process being almost impossible with large quantities of juice owing to the frothing induced by aqueous solutions of the sugars. The variations are, in most cases, probably due to mechanical errors introduced during pressing, by the difference in the state of the tissue of the samples. It is not at present possible to regard them as significant.

TABLE IV.

Nitrogen in Expressed Apple Juice.

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
As percentage of fresh weight	0.0058	0.0084	0.0065	0.0120	0.0102	0.0065	0.0063	0.0079	0.0067	0.0059	0.0067
As percentage of total N.	20	28	22	33	31	22	25	30	28	22	27

The general conclusion is that the nitrogenous substances in the apple are all of a protein nature. Of this protein 80 per cent. is non-extractable by water, while of the 20 per cent. extracted by pressing and washing only a very small fraction is in some soluble form, the rest being colloidal. The nitrogen is therefore assumed to be derived almost entirely from protoplasmic protein, there being, however, a trace of some soluble form of nitrogen.

IV. NITROGEN CHANGES DURING STORAGE.

Three sets of apples were examined—Sweet Alford from Long Ashton (Bristol) and Bramley's Seedling from Spalding, stored at 3° C. and analysed at intervals of about five weeks; and Bramley's Seedling from Spalding, stored at 1° C. and analysed at fortnightly intervals. The results of the analysis are shown graphically below; the dotted lines give percentages referred to original fresh weight, and the continuous lines to weight at time of analysis. The figures for the Bramley's Seedling stored at 1° C. and Sweet Alford are results of duplicate analyses on a mixed sample of ten apples: those for Bramley's Seedling stored at 3° C. are the means of ten determinations on single apples. Both sets of Bramley's Seedlings showed varying degrees of internal browning by the end of the storage periods; those at 3° C. being in addition shrivelled and exhibiting some browning of the skin. The Alfords showed a considerable amount of 'scald' and were much shrivelled, but had no signs of internal browning.

These curves show considerable fluctuations and a downward trend. As already stated (p. 100), the fluctuations must be neglected owing to the large size of the probable error of the analytical results. The very definite downward trend of the results indicates clearly a fall in the amount of nitrogen as time proceeds. The maximum total fall in the case of Bramley's Seedling at 1° C. and 3° C. is in ten months 20 per cent. of the original amount present. The temperature difference of 2° C. has no apparent effect on this loss. No data as to the original fresh weight of the Alfords were available, but the loss of weight during storage must have been considerable, and thus the change in nitrogen larger than the curve E indicates.

The question arises as to the nature of the fall in nitrogen determined by the Kjeldahl method. A loss in the form of ammonia would seem

a possibility, but against this we have the fact that the sap is acid, and also the ammonia should be detectable in the juice. Furthermore, if the proteins are being degraded into ammonia some of the intermediate products should appear in the juice; there is, however, no increase in the nitrogen of the juice during storage. The absence from the juice of such degradation products suggests some other form of protein decomposition.

It may be that a process of slow protein oxidation is in progress, leading to the production of nitrate or nitrite which would not have been estimated in the Kjeldahl method. If nitrite were produced it would attack the amino groups of the remaining protein and elementary nitrogen would be eliminated. If this suggested oxidation does occur owing to inability of the cells to use carbohydrate, or increased activity of the oxidases, or other reason, it seems possible that the poisonous action of nitrous acid in removing amino groups, and thus changing the nature of the protoplasm. may be an important factor in the production of physiological breakdown in the tissue. The nature of the nitrogen changes is being further investigated.

V. Relation of Nitrogen to Other Constituents.

A study of ten individual apples (Bramley's Seedling from Spalding stored at $1°$ C.) was made on two occasions (April 30 and May 28, 1924) during storage, in order to obtain some relationship between respiration and chemical composition. The nitrogen content and acidity (titration value) were determined, and the respiration data supplied by Dr. Franklin Kidd and Dr. Cyril West working at the Low Temperature Research Station, Cambridge. The results are shown in Table VI, and the relation between nitrogen content and respiration exhibited graphically in Fig. 2.

TABLE V.

Analysis of Single Apples.

Bramley's Seedling (Spalding) stored at $1°$ C.

	April 30th.				May 28th.		
	Resp. Rate. c.c. CO_2 per 10 Kilo-hrs.	$N\%$	Titration.		Resp. Rate. c.c. CO_2 per 10 Kilo-hrs.	$N\%$	Titration.
Apple I	27.8	0.0271	14.35	Apple XI	24.3	0.0208	16.1
„ II	23.5	0.0257	15.8	„ XII	26.2	0.0231	16.2
„ III	22.1	0.0119	13.8	„ XIII	27.4	0.0238	14.6
„ IV	27.8	0.0209	12.7	„ XIV	20.5	0.0178	14.75
„ V	26.8	0.0227	10.75	„ XV	22.9	0.0204	11.3
„ VI	28.2	0.0280	14.35	„ XVI	26.7	0.0265	6.9
„ VII	21.9	0.0174	16.9	„ XVII	26.5	0.0273	10.25
„ VIII	29.2	0.0354	14.95	„ XVIII	24.3	0.0212	9.95
„ IX	24.8	0.0179	15.65	„ XIX	28.2	0.0250	10.8
„ X	27.7	0.0278	14.55	„ XX	24.1	0.0232	10.7
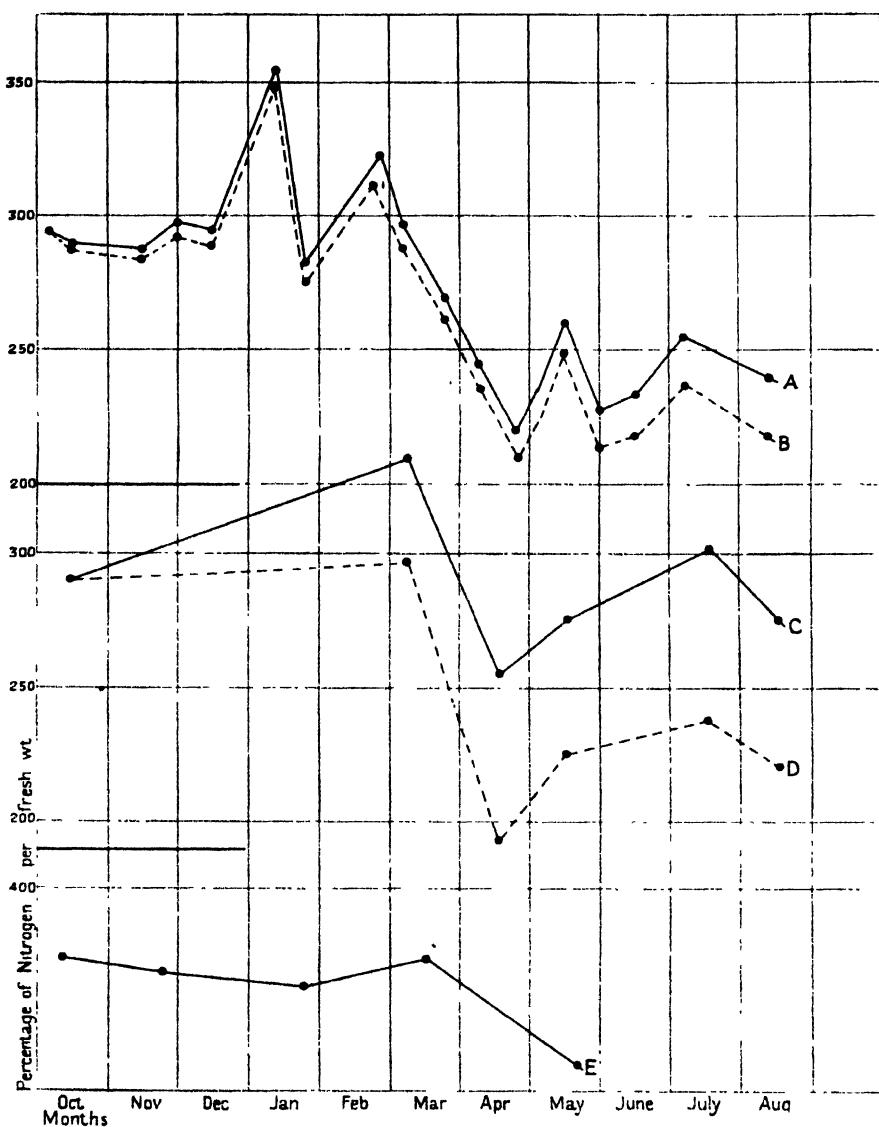
FIG. 1. Changes in nitrogen content of apples during storage.
Bramley's Seedling (Spalding)—

4° C.	A.	Nitrogen referred to fresh weight at time of analysis.
	B.	,, ,, to original fresh weight.
3° C.	C.	,, ,, to fresh weight at time of analysis.
	D.	,, ,, to original fresh weight.

Sweet Alford (Bristol).
    E. Nitrogen referred to fresh weight at time of analysis.

It is seen that a high nitrogen content is in general accompanied by a high respiration rate. This suggests that the quantity of protoplasm determines the rate of oxidation in the apple. It seems, however, that during the storage life of the apple other factors besides the nitrogen loss come into play which also tend to diminish the respiration rate, for in Fig. 2 it will be seen that for a given nitrogen value the respiration rate is lower at the later date than at the earlier.

It is interesting to note that the Bramley's Seedlings obtained from this
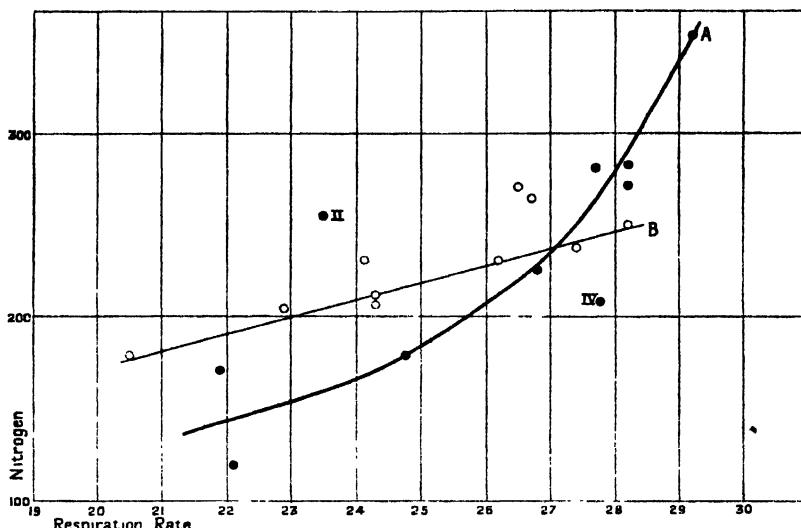
FIG. 2.   Relation between nitrogen content and rate of respiration of single apples.
A. Analysed April 30.   B. Analysed May 28.

source have on an average the lowest nitrogen content observed in this variety or any variety of apple studied, and that they also exhibit particularly good keeping properties. This suggests that the low rate of oxidation set by the low nitrogen content retards the onset of break-down.

In considering the effect of other factors than nitrogen content on the rate of respiration it is also of interest to note in Fig. 2 that the points for apples II and IV, which lie markedly off the curve A, have respectively a high acidity associated with a high nitrogen content (II), and a low acidity and low nitrogen content (IV), the converse being usually found.

It is not of course possible to draw any definite conclusions from these limited data ; it would seem probable, however, that as acid is presumably used in respiration the acidity and oxidizing powers are inversely related ; marked abnormalities in the acidity would, however, affect the normal nitrogen-respiration relation.

Further experiments are being carried out on fruit at earlier stages of its storage life in order to elucidate these relationships.

SUMMARY.

The method of determination of nitrogen in apples and the order of accuracy of the results is described. The percentage varies from 0·02 to 0·08 per cent. of the fresh weight.

The probable error of the mean of ten determinations of single apples is 4·5 per cent., indicating that occasional non-significant differences of 20 per cent. may occur.

The variations in the nitrogen content of single apples of the same variety may be as much as 0·55 per cent.

The nature and distribution of nitrogenous substances is described. The nitrogen is shown to be in the form of protein, of which only a trace can be extracted in a truly soluble form; the determined nitrogen is regarded as a measure of the protoplasm present.

The nitrogen, as determined by the Kjeldahl method, is found to decrease during storage of the apple; since no protein degradation products were found in the juice, a process of protein oxidation is suggested to account for the change.

A correlation is observed between the nitrogen content, acidity, and rate of respiration of single apples, a high nitrogen value usually being associated with low acidity and high respiration rate.

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Chemical Studies in the Physiology of Apples.

III. The Estimation of Dry Weight and the Amount of Cell-wall Material in Apples.

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With three Figures in the Text.

IN the investigation now being carried on at the Imperial College of the nature of the chemical processes leading to the break-down of the apple during storage (4, 5), a rapid method of finding the dry weight of a large number of samples was required. The dry material of tissue of this kind consists of 80–90 per cent. water-soluble substances, chiefly sugars and acids, and 10–20 per cent. cellulose, pectin, and other insoluble substances. Dry-weight data should afford a simple means of comparing the amounts of material present in different samples and of tracing losses due to respiration. Also by determining the amount of cell-wall material as well as total dry weight, some measure of changes in the concentration of the sap of the apple can be made.

    The percentage of sugar is very high, both absolutely and in relation to other solids present. As it is so high and such a small amount is used in respiration it is improbable that such small changes of concentration of sugar as occur have any marked effect on the metabolic activity of the fruit other than a concentration effect, though changes in the relative proportions of different sugars present may be significant. It seemed desirable, therefore, to find a method for estimating the amount of cell-wall material concurrently with the dry weight, so that smaller changes in this quantity should not be masked by the variations of the sugars. The possible influence of changes in the nature of the cell-wall on the water relations of the cell and the possible use of the amount of cell-wall as a measure of cell size indicate the importance of such an estimation.

Further, a dry-weight method of obtaining a measure of the total carbohydrate effects an economy of material as well as of time. The dried material can be used for other purposes, a fact of importance when dealing with single apples, for which data as complete as possible are required.

The present paper deals with:

(1) The determination of dry weight and its correlation with the percentage of solids present as calculated from the density of the juice.

(2) The determination of water and alcohol-insoluble material.

(3) The application of these methods to the analysis of stored apples.

I. DETERMINATION OF TOTAL DRY WEIGHT.

No very satisfactory method apparently exists for determining accurately the dry weight of plant material containing a large amount of both sugars and water. Link and Tottingham (3) examined various methods of desiccation for tissues containing a high percentage of carbohydrates. They concluded that changes occurred to a greater or less degree under all the conditions they tried, and they finally recommend drying *in vacuo* at 80° C. This was quite impracticable in the present case, owing to the bulk of material to be dried and the length of time required to dry such quantities at low pressures. Other methods being available for the detailed analysis of the sugars, it was decided to try a high temperature. which would inhibit loss from enzyme action and respiration. and ordinary pressures. The nature of the changes in the sugars, provided the absolute loss was not appreciable, could be disregarded.

Comparison of these conditions with others less liable to produce changes in the tissue show that not only can reliable comparative results be obtained by accurately reproducing the conditions of experiment, but satisfactory estimates of the dry weight can be made.

The method in use is to dry 50-grm. samples of the pulp, cut finely by hand, in a ventilated oven at 100° C., using about the same amount of material for each experiment. The drying is continued for thirty-six hours for reasons explained below. The samples do not reach a constant weight even after sixty-five days' drying, but the results are nevertheless strikingly uniform.

For any period of drying the difference between the greatest and least values of ten estimations does not exceed 3 per cent. of the mean result.

Table I gives one of several sets of results.

The continued loss under these conditions is of course not one of simple dehydration. After thirty-six hours' drying the samples reached a stage of steady rate of loss, which was maintained until the total time of drying was 130 hours, when the rate slowly decreased again. Charring was very apparent by this time. The period 36–130 hours indicates a process of

chemical change. Loss of water of composition, due to slow production of caramel together with some oxidation, is probable. If simple dehydration were occurring, the rate of loss would continue to diminish. The thirty-six-hour period was chosen therefore as a basis for comparison, as it apparently marks the completion of dehydration.

TABLE I.

Dry Weights 50-grm. Samples, dried at 100° C. for Thirty-six Hours.

% *Dry Weight.*

1.	16.21
2.	16.15
3.	16.27
4.	16.15
5.	16.37
6.	16.34
7.	15.99
8.	16.22
9.	16.16
10.	16.19

16.21 ± 0.07

The rate of loss with time of drying is shown by the following two sets of figures taken from a series of ten estimations of 50-grm. samples of Australian apples (variety unknown).

TABLE II.

Rate of Loss of Samples with Time of Drying.

Period of Drying.		Wt. after Drying.		Loss of Wt. in Grm.	
		A.	B.	A.	B.
1–18	hours	8.4158	8.3162	41.5842	41.6952
18–24	,,	8.3048	8.2231	0.1060	0.0931
24–30	,,	8.2205	8.1384	0.0843	0.0847
30–36	,,	8.1368	8.0755	0.0637	0.0629
36–42	,,	8.0741	8.0179	0.0627	0.0576
42–48	.,	8.0091	7.9670	0.0650	0.0500
48–54	,,	7.9498	7.9116	0.0593	0.0554
54–60	,,	7.8758	7.8512	0.0740	0.0603
60–128	,,	7.4021	7.5109	0.4737	0.3403
128–180	.,	7.1986	7.2114	0.2132	0.2995
180–225	,,	7.0485	7.1084	0.1501	0.1030
225–269	,,	6.9530	7.0033	0.0935	0.1031
269–313	,,	6.8798	6.9250	0.0732	0.0803

These results are plotted graphically below (Fig. 1), together with others of a similar type obtained with three other varieties of apple; they indicate the general nature of this drying process. The varieties employed were Bramley's Seedling (Spalding), Sweet Alford (Bristol), and Dymock Red (Bristol).

Two sets of experiments were carried out to confirm these results. The first attempt, to eliminate the effect of prolonged heat by heating a series of samples at 100° C. for an hour to destroy enzymes, and then at 30° C. in a current of air, was not successful.   In this case a dry crust formed on the outside which prevented desiccation of the inside ; as a result the material was attacked by moulds.
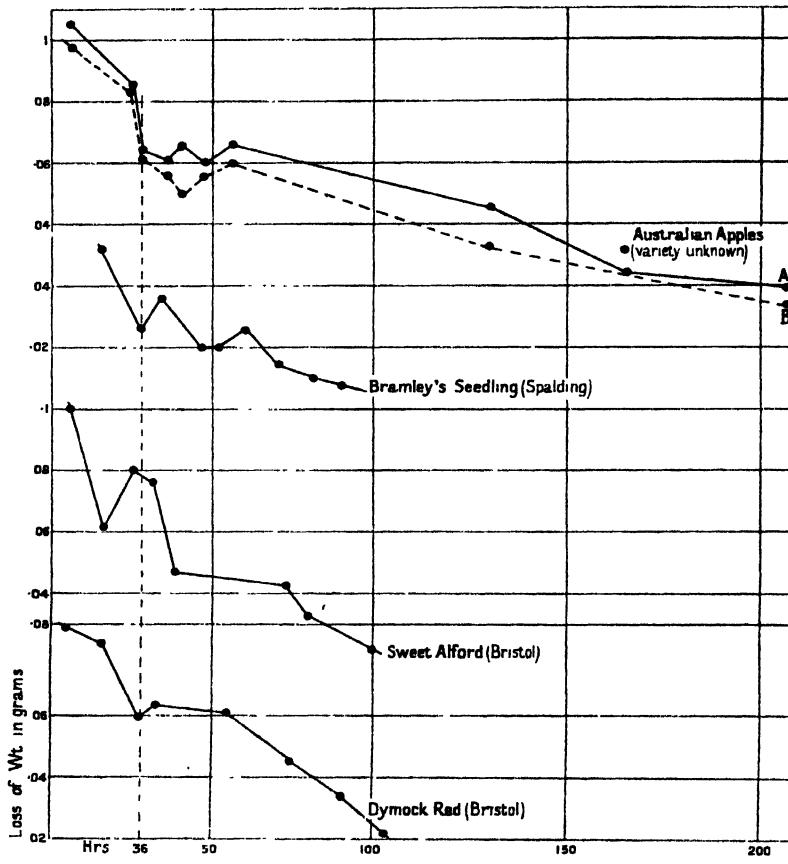
FIG. 1.   Loss of weight of apple pulp during drying.

Secondly, to avoid oxidation and lessen the changes in the sugars, the drying was effected *in vacuo* at 50° C.   A small sample was placed in a weighed test-tube which was inserted into a wider tube connected with the pump and enclosed in a steam jacket.   When exhausted a steady temperature of 50° C. was maintained by keeping the water boiling gently and condensing with a long air-condenser.

Drying by this method, the rate of loss of weight diminished steadily, and after ninety hours a constant weight was reached which agreed remark-

ably well with that obtained by drying at 100° C. for thirty-six hours. Typical results are shown below (Table III).

TABLE III.

Bramley's Seedling.		*Sweet Alford.*	
100° C. *for* 36 *hrs.*	*In vacuo at* 50 °C. *for* 90 *hrs.*	100° C. *for* 36 *hrs.*	*In vacuo at* 50° C. *for* 90 *hrs.*
11.10	10.96	12.93	13.09
10.87	10.79	12.97	13.12

This agreement indicates that losses due to oxidation or caramelization are not important in the first thirty-six hours, since these changes are precluded in the second set of conditions. Thus a true measure of dry weight is obtained by the high temperature method.

Errors arising from Variations in Individual Apples.

These errors were investigated by a series of analyses of single apples. The fruit used was Bramley's Seedling from Long Ashton (Bristol) and from Spalding, both stored at 3°.

These sampling experiments show that the probable error of the mean of ten results is about 1.5 per cent. The order of variation is shown in Table IV.

TABLE IV.

The Dry Weights of Single Apples.

Bramley's Seedling from—

1. *Bristol.*	2. *Spalding.*	
Jan. 29.	*March* 6.	*April* 14.
12.08	10.82	9.75
11.96	10.52	9.80
11.45	10.18	9.35
11.29	10.16	10.90
11.21	10.20	9.73
13.44	10.04	10.56
12.86	10.56	9.45
12.05	9.76	9.10
12.54	9.88	10.82
10.90	11.04	9.63
Mean 11.88 ± 1.43	10.31 ± 0.80	9.66 ± 1.1

Such a probable error (1.44 per cent.) shows that differences between mixed samples of ten apples should be about 5 per cent. to be significant.

Relation of Dry Weight to the Density of the Juice.

In view of these results it is difficult to believe that changes in the sugars due to heat affect the total dry weight materially. In order to obtain further evidence of the accuracy of the method as giving a true measure of dry

weight and not merely a comparative one, the figures were compared with the sum of the alcohol-insoluble residue and the solids per 100 grm. of pulp calculated from the density of the juice. Instead of the figures agreeing within known experimental errors, a discrepancy was found amounting in some cases to nearly 20 per cent. of the observed dry weight; the calculated figure being higher.

The possibility of a constant error in the dry-weight estimation—owing to the accepted end-point marking the end of a definite chemical change and not that of dehydration—is small. Complete caramelization would not be sufficient to give an error of this magnitude even if such were possible at the temperature employed. The recognized temperature for this process is 170° C.; no doubt it occurs slightly at lower temperatures, especially if heating is unduly prolonged, but the comparative experiments described eliminate the possibility of this loss being significant during the first thirty-six hours.

Loss of CO_2 by oxidation during drying might occur. Attempts to detect evolution of CO_2 by drawing a current of CO_2 free air through the sample during drying and passing the air through baryta solution produced negative results.

The juice for density determinations was extracted by freezing and pressing out the killed tissue (1). The density was determined with a 10 c.c. specific gravity bottle, no correction for temperature being made. The errors introduced by this cause, together with those obtained by using the formula $\dfrac{D - 1 \cdot coo}{3 \cdot 85}$ to calculate the solids per 100 c.c. in a mixture such as the juice, by failure to remove all solid particles before determining the density, and by heating the juice to sterilize it, do not exceed 5 per cent.

Examination of artificial mixtures of sugars, malic acid, and pectin in the concentrations found in the juice show that an error of about 3 per cent. occurs by using this formula. The results are too low and are not affected by previous exposure of the solution to cold (1° C.) and heat (80° C.), the extremes to which the juice is subjected. With the exception of the error due to the use of the formula, the errors from the causes enumerated above tend to give a high value; hence the various known sources of error compensate one another.

These observations point to the existence of a third factor vitiating the calculation of dry weight from density measurements. It seems probable that the juice contains a volatile constituent of high density which is removed during the drying process. This view is supported by examinations of the juice and of the effect of glycerine on the densities of artificial mixtures and by observations of the relation between density and dry weight throughout the storage period.

To obtain data of the density and dry-weight changes with time Bramley's Seedlings stored at 1° C. and at 3° C. were examined. In the

first case the dry-weight and density curves were remarkably parallel until May, when a sharp fall of density occurred, not accompanied by a similar fall in the dry weight. In the second case the curves approached one another throughout the season, the rate of fall of density being greater than that of dry weight. The results are shown in Fig. 2.

In Fig. 3 the data for Bramley's Seedling stored at 1° C. shown in Fig. 2 (II *a*) are used to plot against density, not only the observed dry weight but also the dry weight calculated as described on p. 114. It shows that the
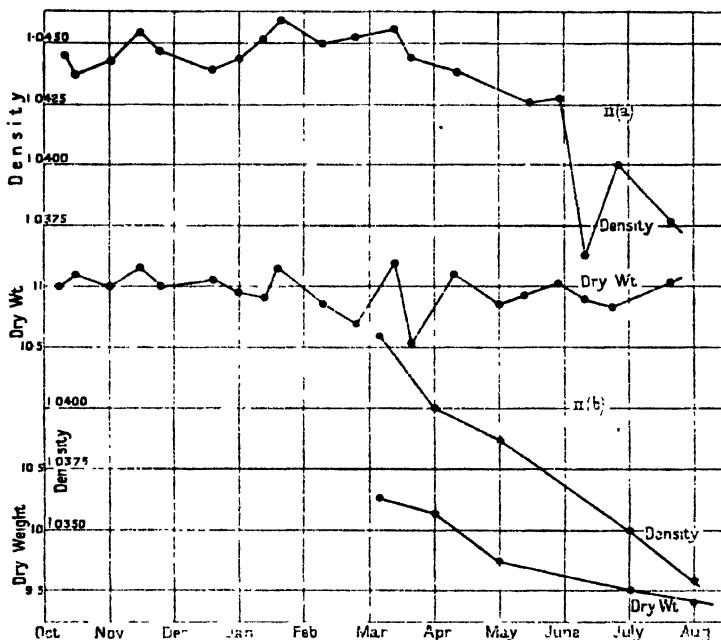
FIG. 2. Changes of dry weight and density of the juice of Bramley's Seedling apples during storage. II (*a*). Stored at 1° C. and analysed fortnightly. II (*b*). Stored at 3° C. and analysed every five weeks.

discrepancy between the two sets of dry weights falls off as the density decreases; i.e. in time. In this experiment the discrepancy disappeared between July and August.

The existence of some substance gradually decreasing in amount as the storage life progresses, and the rate of loss of which is determined by the temperature of storage, seems therefore the probable explanation of the anomaly.

The addition of small quantities of glycerine to artificial mixtures caused sufficient increase in density to bring the calculated values well above the actual amount of sugar present. The amount required for the maximum

difference found with the juice is 0·75 c.c. per 100 c.c. Juice from apples analysed in July had its density decreased 4 per cent. by distilling *in vacuo* to half the volume and adding distilled water to make up the original volume. This corresponds to about half the difference observed at this time. The presence of a higher alcohol thus offers a reasonable explanation of the facts, and attempts to isolate such a substance are in progress.
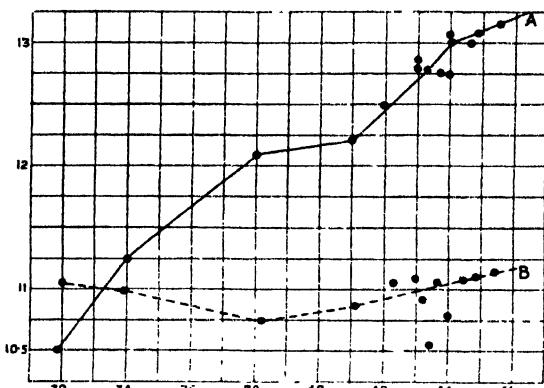
FIG. 3. Changes in the relation between observed and calculated dry weights with variations in density of the juice of Bramley's Seedling apples. A. Dry weight calculated from the density of the juice. B. Dry weight formed by drying at 100° C for 36 hours.

II. CELL-WALL MATERIAL.

The main problem in finding a method of estimating the amount of cell-wall material has been to reduce the amount of hydrolysis to a minimum during extraction of the soluble substances. Some of the constituents of the wall are undoubtedly very readily hydrolysed, and, with such a high percentage of soluble material to be removed, the process of extraction is of necessity prolonged; some conversion of carbohydrates originally in the wall is therefore unavoidable.

Three methods have been tried, of which two give results agreeing well with each other; and these are now taken to give a fair measure of the cell-wall content. At least, these results give quantities which can be compared, and since little is known about the actual constitution of the cell-wall, this is all that can be attained at present.

Extraction with water. The first attempt was based on the method of Carré (2) for the extraction of soluble pectin from apple tissue. The advantage of this method was that the frozen pulp could be used and the heat effect was eliminated, but the difficulty of removing the residues from the pressing cloth and the mechanical errors involved before extraction was complete rendered it inapplicable.

To therefore avoid the necessity of pressing, the dried tissue was employed, and since this could be readily ground to a fine powder the problem of disintegrating the material was eliminated. Continuous washing with cold water removed sugars, acids, and soluble pectin, leaving insoluble pectin, cellulose, and possibly compounds of cellulose, which were weighed.

The material was dried for eighteen hours, then ground and placed in tared weighing-bottles and dried for a further period of eighteen hours. It was then transferred to a filter-paper, and washed until free from sugars, acids, and soluble pectin. About 2 litres of washing water were necessary for the quantities used.

The residue was washed off the filter-paper into small evaporating basins, and transferred to Gooch crucibles for drying and weighing. The order of agreement of results is shown in Table V.

TABLE V.

	Weight of Ground Material.	*Insoluble Residue.*	*Percentage of Total.*
1.	1·5333	0·2360	15·4
2.	1·1623	0·1815	15·6
3.	1·2867	0·2010	15·6
4.	1·7220	0·2715	15·8
5.	1·8141	0·2840	15·7
6.	1·4423	0·2290	15·9
7.	1·5741	0·2418	15·4
8.	1·5518	0·2453	15·8
9.	1·8385	0·2890	15·7
10.	1·2988	0·2030	15·6

Mean 15·67 ± 0·04

Further investigations into the effect of prolonging the time of drying proved that a large increase in this produced a decreasing amount of soluble pectin and an increasing amount of insoluble material. Up to forty-eight hours, however, these variations do not exceed a maximum difference of 4 per cent. (see Table VI). Beyond this point the changes were out of all proportion to the total loss of dry weight. In the variety of apples used the insoluble material is doubled after 160 hours. The uniformity of the drying action was again emphasized by the agreement of estimations at any given interval. The errors introduced by failure to reproduce the drying conditions are shown by the tables (VI and VII). Table VI gives the variations up to forty-eight hours. The samples were dried and weighed at intervals of six hours after the first thirty hours. Two were removed, and extracted, as described, at each six-hour interval. The results are calculated as percentages of the weight of the sample at each interval, and show that over this range any chemical change due to heat does not introduce a greater error than that existing between samples analysed under strictly analogous

conditions. Table VII shows analyses of same type over a range of time of drying 30–160 hours. The insoluble material reaches a constant weight after drying overnight.

TABLE VI.

Effect of Time of Drying on Water-insoluble Material.

Time of Drying.	30 *hrs.*	36 *hrs.*	42 *hrs.*	48 *hrs.*	54 *hrs.*
Dry wt. as % of dry wt. after 30 hrs.	100	99·12	97·88	97·61	96·75
Insoluble material calculated as % of dry wt. at time of extraction	13·31	12·95	13·07	13·06	13·29

Except for the 54 hr. sample the results are the mean of two samples.

TABLE VII.

Australian apples, 3 % *soluble pectin.*		*Early picked English apples. Trace starch, no soluble pectin. Insol. mat. % referred to total dry wt. after*			
Hrs. drying.	*Insol. material* (%).	*Hrs. drying.*	48 *hrs.*	75 *hrs.*	120 *hrs.*
28	11·5	48	32·7	—	—
34	11·45	75	44·38	46·01	—
53	11·6	120	52·89	55·37	56·47
76	13·29	144	55·67	58·15	59·45
100	13·82				
144	25·0				
168	31·2				

The heat effect is not marked, therefore, until after about fifty hours, so that the dry-weight material obtained by the method described can be used to obtain trustworthy results.

Any attempts to accelerate the washing process by using hot water or by heating with a small bulk of water and a trace of acid or alkali produced very irregular results and much lower than the above, marked hydrolysis having occurred.

Autoclaving for twenty minutes was also tried on the fresh tissue to macerate it and so avoid the long heating during drying, but this, too, gave very unsatisfactory results.

Extraction with alcohol. The third method consisted of extraction of the fresh tissue with alcohol. To obtain all the alcohol-insoluble material and avoid the necessity of pressing, the finely cut pulp was soaked overnight in 98 per cent. alcohol, and then filtered off and extracted in a Soxhlet extractor for twenty hours with fresh alcohol, when the weight of the

residue was found to be constant. Addition of alcohol to the first extract did not effect precipitation of material which might be soluble in the water-alcohol mixture and not in pure alcohol. The residue was then dried to constant weight (sixteen to eighteen hours). The figures obtained agreed well.

Comparison of the values for water-insoluble material and alcohol-insoluble material showed that the relation between the two estimations varies with time. In immature apples picked in June and July the water-insoluble material was about double the alcohol-insoluble. This divergence decreased until at the normal picking time the two figures agreed. As time progressed the water-insoluble material diminished more rapidly, and by the end of the season was 4 per cent. lower than the alcohol-insoluble. It was thought that this difference might be due to the soluble pectin which is washed out by the water method. Estimation of soluble pectin in the water extract by the method of Carré and Haynes (2) was, therefore, carried out on a sample of apples late in the season, when the soluble pectin was high. The figures for this comparison, together with a hot-water extraction experiment, are shown below (Table VIII).

TABLE VIII.

	Alcohol Extraction.		*Water Extraction.*				
	Insol. material		*Insol. material*		*Cold Soluble pectin*	*Total*	
	in fr. wt.	*in dry wt. (calculated).*	*in fr. wt.*	*in dry wt. (a).*	*% in dry wt. (b)*	*fr. wt. (calculated).*	*dry wt. (a + b).*
1.	1.825	19.11	1.742	18.24	3.56	2.08	21.8
2.	1.840	19.25	1.763	18.46	3.61	2.11	22.07
3.	1.820	19.06	1.692	17.72	3.68	2.04	21.40
4.	1.822	19.08	1.793	18.36	3.97	2.13	22.33
5.	1.859	19.46	1.719	18.00	3.87	2.08	21.87
6.	1.836	19.22	1.726	18.07	3.57	2.07	21.64
7.	1.826	19.17	1.754	18.37	3.55	2.09	21.92
8.	1.825	19.11	1.688	17.80	3.49	2.03	22.29
9.	1.833	19.19	1.704	18.54	3.68	2.12	22.22
10.	1.854	19.41	1.719	18.00	—	—	—

Hot-water Extraction.

% insoluble material dry wt.	% soluble pectin.	Total.
13.50	5.40	18.9
13.43	9.01	22.47
13.57	5.31	18.08
13.51	6.44	19.95
13.28	7.89	21.17

These figures show that the whole difference between the results is not due to soluble pectin. The increasing quantity of this substance during storage accounts for some of the difference, but since the sum of the water-

insoluble material and soluble pectin is consistently higher than the alcohol-insoluble at this time (July), there is evidence of some alcohol-soluble material produced during storage; this has been confirmed by direct observation, but the substance has not yet been identified. The hot-water extraction was effected by washing the ground powder with half a litre of water and then boiling gently for an hour with fresh water. The hydrolytic effect is well marked, and it is of interest to observe that it seems almost restricted to the production of soluble pectin.

Both the cold-water and alcohol extraction methods can therefore be used and give concordant results, the difference between them affording a rough measure of soluble pectin. The advantage of the alcohol method lies in the fact that it requires less manipulation; the elimination of sampling error effected by the grinding of dried material is important, especially late in storage life.

When for purposes of comparison a considerable number of estimations have to be made the alcohol method is the more useful, especially early in the season, when the apples are less variable. For routine work throughout the year the water method is preferable.

Changes in dry weight during storage. These methods have been used to follow the changes of dry-weight and cell-wall material in Bramley's Seedling apples stored at 1° C. and 3° C.

At 1° C. the percentage of dry weight, referred to fresh weight at the time of analysis, remained remarkably constant, and the variations were within the sampling error of the estimation (Fig. 2). This means that the concentration of the sap is maintained during storage at this temperature and this humidity, although the nature of the solutes doubtless varies.

At 3° C. the temperature and humidity were such that the rate of loss of dry weight was sufficiently increased, relative to water loss, for the dry-weight fresh-weight relation to decrease with time (see Fig. 2). It is hardly to be expected that the concentration in question would be maintained over any range of temperature, and the conditions of temperature and humidity in the 1° C. store must be regarded as a special case of the relation governing loss with time of water and carbohydrates at varying temperatures. An extended investigation is in progress on the effect of greater temperature differences.

The rate of decrease of the cell-wall material follows the same lines as the total dry weight; a slight decrease in its ratio to the fresh weight being observed at the end of the storage life at 1° C.; this diminution is more marked at the higher temperature, which no doubt accelerates hydrolysis.

Whether cold retards the rate of loss of dry weight by merely lessening the rate of chemical reactions or by altering the nature of the reactions, or by both, is not yet established, but it seems probable that the retardation is selective.

Summary.

Methods of determining total dry weight of apples were investigated, and one in which material is heated for thirty-six hours at 100° C. adopted.

The dry weight obtained by this method is found to be lower than that obtained by calculation from the density of the juice.

This difference decreases during storage and is attributed to the presence of a volatile higher alcohol, whose amount decreases with time.

Methods of determining the amount of cell-wall material by water extraction and alcohol extraction are described and discussed.

These methods are applied to the determination of the changes in dry weight and cell-wall material during storage of the fruit.

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On Carpel Polymorphism. I.

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With eighty-three Figures in the Text.

I. INTRODUCTION.

THE view, recently advanced,[1] that the notion of one uniform pattern of carpel throughout the group of Angiosperms was not borne out by the facts, and could no longer be maintained, followed as the logical outcome of the study of certain exceptional fruits which appear not infrequently in many different genera of the Cruciferae.[2] As the structural relations of these atavistic fruits, as they proved to be, gradually became clear it became simultaneously evident that they could not be brought into harmony with current views, while to dismiss them as monstrosities was merely to beg the question. The appearances in this and other families investigated strongly suggested the occurrence of carpels of different structural types, fulfilling different functions. Extension of the field of inquiry has since fully confirmed this interpretation and afforded proof that throughout the whole range of flowering plants we meet with reduction in number and ' consolidation ' of the members of the gynoecium, the latter process having resulted in the production of certain definite carpel forms and having been accompanied by a redistribution of the carpellary functions.

In the preliminary account of these observations three types of carpel are distinguished, viz. the hollow or valve type, probably the most primitive and hitherto the only one recognized, the semi-solid or pseudo-valve, and the solid form.[3] It was shown that this interpretation of the structure of

[1] See A Reversionary Character in the Stock (*Matthiola incana*) and its Significance in regard to the Structure and Evolution of the Gynoecium in the Rhoeadales, the Orchidaceae, and other Families. Ann. Bot., vol. xxxvii, p. 451, 1923.

[2] Ibid., p. 464, foot-note 2.

[3] Ibid., pp. 460, 476.

the gynoecium enables us to appreciate many anatomical features which before had appeared devoid of significance, and to account for others of a more puzzling nature, such, for example, as the commissural stigma, an anomaly characteristic of each of the families so far considered (viz. those belonging to the Rhoeadales and Orchidaceae) and only explicable on the orthodox view by means of a series of entirely unfounded assumptions which are now shown to be unnecessary.

In pursuing this line of investigation farther, the original intention had been to deal in the first instance with the various other families and scattered genera exhibiting the above-mentioned anomalous character. As it soon became clear, however, that carpel polymorphism was a phenomenon of very general occurrence and not necessaiily associated with a particular stigma position, this procedure would have unnecessarily restricted the choice of material, and was not adhered to. With the whole field unexplored one might freely select illustrative examples where one would, or as suitable material offered. The case proved beyond question for a further number of widely scattered types, the necessity of reviewing the whole Angiosperm group, family by family and genus by genus, from the new standpoint regarding carpel evolution would be manifest.

II. The Monomorphic Carpel View.

According to current conceptions, the one-carpelled ovary is formed of a single specialized leaf folded lengthwise and inwards so that the meeting edges cohere and give rise to marginal placentae. In the ovary composed of more than one member each carpel differentiates in contact with its neighbours, with the result that the contiguous sides of adjacent carpels are not delimited the one from the other. The syncarpous gynoecium thus formed may show parietal, axile, occasionally free central, or, more rarely still, superficial placentation. In the case of parietal placentation the ovary may be without any partitions, or it may show any degree of chambering short of the true multilocular condition according to the degree of intrusion of these undelimited carpel sides into the ovary cavity ; or as it would appear to be more correctly described in many cases, *according to the degree to which these incomplete septa are withdrawn from the centre as development proceeds.* If the undelimited sides of the carpels do not become detached from one another at the centre, but continue to form a common mass of tissue, the actual edges alone separating and re-entering to form the placentae, true axile placentation results. The free-central arrangement, which on the monomorphic view necessitates the assumption that the several placentae separate each from the body of the carpel of which it is part at or near its base, and together form a central column disconnected for most of its extent and sometimes entirely from the ovary wall, has

always presented a certain difficulty.[1] The same may be said of super-ficial (scattered) placentation, of which no satisfactory explanation has so far been put forward. Lastly, besides the above-mentioned types of gynoecium there is the category in which the carpels are held to be disposed around a prolongation of the axis itself. Whatever the arrangement and degree of separation of the carpels or the mode of placentation, however, the carpel member is conceived as invariably having the same morphological character, viz. that of an expanded and folded leaf. But, as has been pointed out earlier, there are from this standpoint many difficulties and anomalies to be got over which, as has already been indicated and is more fully shown here, are easily brought into harmony on the theory of poly-morphism. For, in addition to the stumbling-block of the so-called *com-missural* stigma with its attendant features of *double* sutural contour lines, *false* partitions, and *detached* placentae, there are cases of the occurrence of more styles and stigmas than correspond with the (accepted) number of carpels, of the development of subsidiary loculi, and of the regular formation of dimorphic fruits.[2] Again, there are cases where the monomorphic carpel conception is definitely at variance with the evidence furnished by the modes of venation and dehiscence, and with the law of alternation of succes-sive whorls. as in certain cases of obdiplostemony.

III. The Polymorphic Carpel Theory.

On the polymorphic carpel view, the phenomenon which is recognized as taking place frequently in the other floral whorls (calyx, corolla, and androecium) is held to be of common occurrence also in the gynoecium, in which, contrary to received opinion, several types of carpel are conceived as having arisen in the course of evolution with accompanying separation and redistribution of the carpellary functions. Where the type of carpel which we may take to be the most primitive alone is present, each fully developed member fulfils a threefold role—receptive, reproductive, and protective. But when two different types occur together there is division of labour, though no one of these three functions is absolutely restricted to one particular structural pattern. The three forms so far distinguished may be more definitely characterized as follows:

1. The *valve* carpel retains more or less the typical leaf form. A mid-rib is generally visible externally, and in dry fruits is often a conspicuous feature. Reticulate venation of the pinnate type is usual, but an approach to the palmate form is met with occasionally, as e. g. in *Coptis aspleni-*

[1] Unless it is held, as it is by certain French writers, that the central column in these cases is axial in character.

[2] Discussion of certain illustrative cases coming under these heads will be found in the earlier paper, for which see foot-note 1 on p. 123.

folia, Salisb. (Ranunculaceae) (see Figs. 1–3), and *Butomus*. The scheme of venation, as we shall see presently, is a character of no small importance from the present point of view. Where the valve type of carpel is the only one present it serves all purposes. If a style is developed it is formed by a prolongation of the midrib and bears a single stigma. In the simplest case each carpel edge carries only a single row of ovules, but when the sides of adjacent carpels fail to become delimited at their margins the common placentiferous region may bear several rows, as may be seen, e. g., in species of *Sempervivum, Sedum,* and *Echeveria* (Crassulaceae), all apocarpous types.[1] Dehiscence in the syncarpous capsule composed of valve carpels is septicidal or sutural (*Platystemon, Platystigma*(?) and some species of *Melanthium* belonging to the section Melanthioideae of the Liliaceae). Although in some genera capsules composed of valve carpels have the appearance of dehiscing loculicidally, many such cases on investigation prove not to be genuine instances of splitting of a valve at the midrib, but result either from the separation of semi-solid carpels from an intervening solid carpel, as in Orchidaceae, or from division of the double bundle forming the midrib of a solid or a semi-solid carpel, as in the Garden Tulip and *Fritillaria Imperialis*, L. (see later, pp. 159 and 161). It follows that all cases so described need to be examined afresh from the new point of view.

The *solid* carpel is in strong contrast to the typical valve from which we may suppose it to have been evolved. In its most reduced form it consists merely of a fibro-vascular cord with but few lateral veins and reticulations connecting it with its neighbours, which have in the course of their own expansion, so to speak, engulfed it. In a carpel somewhat less attenuated, this cord may be enveloped in an area of ground tissue proper to itself, with only one or with both epidermal surfaces delimited from the carpels on either side, which in the latter case are thus distinctly marked off from it. In its most fully developed state the solid carpel not only separates completely the radial surfaces of the carpels on either side, but it may undergo a further development, and by projecting into the ovary cavity give rise to a (so-called) false partition (Cruciferae). Or, on the other hand, such a carpel may from its earliest development form a complete septum of this kind at the base of the gynoecium, and then extend radially less and less far towards the centre at successively higher levels until finally it is completely withdrawn, and there remains instead of a radial plate only an undelimited sector of the ovary wall, as e. g. in *Linum*. The solid carpel occurs associated sometimes with true valve carpels belonging either to the same whorl (most Cruciferae) or to a different whorl (four-valved Cruciferae,

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[1] A similar condition is shown in Eichler's diagram of *Platystemon* (Papaveraceae), a syncarpous type, but it would seem incorrectly, for Payer definitely states that the placentae in this genus bear only two rows of ovules, which is the normal disposition when valve carpels cohere merely by their edges, and this certainly appeared to be the case in the specimens I examined.

Primulaceae), sometimes with pseudo-valves (*Fritillaria Meleagris*, L., Orchidaceae), sometimes with several others of the same type (Umbelliferae, *Arachis* and *Scorpiurus* among the Papilionatae, *Castanea, Gentiana*). When solid carpels occur together with valve carpels they are almost always fertile and the valves sterile (most Cruciferae, Primulaceae). Stigmas may then be borne only by the solid members (many Papaveraceae, most Cruciferae) or by both solid and valve carpels (a few Cruciferae, as e.g. *Biscutella*), or by the valves alone (Geraniaceae, Primulaceae). *Paepalanthus* (Eriocaulaceae) presents an extremely interesting case. The supposedly three-carpelled, in reality six-carpelled, ovary shows two forms of stylar prolongations, of which three stand over the fruit valves and are non-functional and three are commissural, two-cleft in some species, and stigma-bearing. We find a similar condition exhibited by *Parolinia* (Cruciferae), while in many forms among the Fumariaceae the single style bears two dissimilar sets of appendages, one representing a pair of functional and the other a pair of non-functional stigmatic processes (well seen in *Platycapnos* (*Fumaria*) *saxicola*, Wilk., but also to be made out in the common Fumitory). The medianly placed vascular tissue of the well-developed fertile solid carpel may appear as a single strand or as twin bundles forming a more or less completely double cord, branches from each bundle supplying one or more rows of ovules on the corresponding side. Herein we have the explanation of the two-cleft character of the style sometimes seen in carpels of this type, as e.g. in *Paepalanthus* referred to above. There remain to consider the cases of combination of solid with semi-solid carpels, but these will be more conveniently dealt with in the account of the latter type.

The *semi-solid* or *pseudo-valve* carpel combines some of the features of both the other forms, and is of equally widespread occurrence. Outwardly it has the valve contour, but it differs fundamentally from the true valve in that the placentae are displaced from the contact edges of the carpels to a position on either side of the centre line. The disposition of the vascular tissue, which also differs from that of the valve, goes hand in hand with the altered arrangement of the ovules. It shows a double central strand (midrib) from which connexions run direct to the funicles, while other branches supply the ovary wall. This type of venation enables the semi-solid carpel to split at maturity down the centre between the two bundles of the midrib, as can be seen in *Fritillaria Imperialis*, L., and in most Papilionatae. This arrangement of the vascular tissue accounts for another feature by which this type, as well as the solid carpel, is distinguished from the valve proper. In this latter type only one style and stigma is borne by each member, but in the stigma-bearing semi-solid type the twin vascular strands forming the midrib are both continued upwards, so that frequently when a style is present it bears two stigma branches (species of *Drosera* (Fig. 38), *Begonia* and *Salix*,

Datisca cannabina, L.), or, if a style is absent, two sessile stigma lobes may sometimes be formed by each carpel, as in the Garden Tulip. Semi-solid carpels are found in combination both with valve carpels (Berberidaceae (*Epimedium*)) and solid carpels (Liliaceae (*Fritillaria Meleagris*, L.) and Orchidaceae), and, so far as observation has yet gone, are then always fertile. When the ovary is composed entirely of semi-solid members, they may either all be fertile (*Begonia*), or half of them may bear ovules and the other half may be sterile, as in *Fritillaria Imperialis*, L. (Liliaceae), and also *Haematoxylon campechianum*, L. (Caesalpinieae)). In a homomorphic gynoecium composed of semi-solid carpels, some, if not all, must of necessity perform the stigmatic function, but in the heteromorphic ovary they sometimes retain this function (*Epimedium*) and sometimes not (*Fritillaria Meleagris*, L.). Usually anchor-shaped in cross-section, with the shaft directed inwards, this type of carpel is able to function protectively in the same manner as the valve.

IV.   CONSIDERATION OF CERTAIN FEATURES OF THE GYNOECIUM IN
RELATION TO CARPEL POLYMORPHISM.

Before proceeding to illustrate the statements set forth above by a more detailed consideration of various genera, it may be permitted to sketch in outline what seems to have taken place in the phylogenetic history of the gynoecium. In primitive forms producing only valve carpels, the several members would first be visible as so many protuberances. The vascular bundles destined to form the midribs would become differentiated, curving outwards and upwards. This development, accompanied by a corresponding 'ballooning' outwards of the ground tissue on either side of a midrib, would lead to the formation of a loculus. As expansion of the now hollow carpels proceeded, the gynoecium would exhibit the apocarpous or syncarpous condition, according as the carpels were from the beginning sufficiently distant from one another to enable each to accomplish this development separately from its neighbours, or were differentiated at points so near together that the development of the sides of adjacent carpels as separate structures could not take place. In the latter case radial plates of tissue would appear, each composed of the common side-wall of two contiguous carpels, the ovary thus becoming plurilocular. The first disposition results in marginal, the second in axile placentation. But we have also to take into account the arrangement termed parietal, in which the ovules are borne on the ovary wall or on radially projecting ribs or plates, which, failing to reach the centre, leave the ovary unilocular. Now such a condition might come about in different ways. As the circumference of the ovary enlarged, these radial plates might gradually thin out and leave the centre and persist as incomplete partitions; or the carpels may

be conceived as abutting on each other, not by their sides, but merely by their edges, which just meet and cohere ; or they may be of such width as not only to meet and cohere, but to project inwards for some distance. But whichever be the mode of origin, the view hitherto taken has been that parietal placentae arise from the margins of expanded valve carpels. From the evidence already brought forward in the preliminary account,[1] and from the further considerations set forth here, it is clear that parietal placentation of this nature is by no means of such frequent occurrence as has been supposed. It has been shown that the Rhoeadales, previously regarded as an order in which this mode of origin of the placentae is exemplified throughout, must now be ruled out almost *en bloc*, as also must the Orchidaceae. Indeed, the only genuine instances among the Rhoeadales appear to be *Platystemon* and possibly *Platystigma*.[2] In the earlier account I had included also *Reseda Luteola*, L., but more favourable material has shown that the ovary is not composed of three valve carpels, but of three solid and three semi-solid as in the other trimerous species. The main distinction between *Luteola* and the other species, therefore, comes to this, that the whorl which is solid in it has become semi-solid in the others and vice versa. As to the development of the many-carpelled plurilocular ovary, it seems preferable to regard it, as stated above (p. 128), as produced by the bulging *outwards* of the *centre* of each carpel, which brings the sides with it, rather than as due to the *inward* protrusion of the *flanks* of each member. When a second whorl of carpels is still retained, the two may arise so nearly at the same level, that as development proceeds they come to have the appearance of constituting only one. a condition with which we are familiar in the case of the other floral members. But the trend of evolution here, as elsewhere, has been in the direction of reduction in number and differentiation of form. The latter process, although of extremely common occurrence, has found expression in but a limited number of ways. Reduction, resulting, we may suppose, from a decline in the *strength* or *period* of developmental vigour, led first to the suppression of the second whorl, a process which we can see in the act of taking place in such forms as *Triglochin palustre*, L. (Scheuchzeriaceae), and *Zanthorhiza apiifolia*, L'Hérit. (Ranunculaceae), where the inner whorl of carpels is becoming smaller and sterile. The inner whorl wholly or largely suppressed, the process was often carried farther, causing the disappearance of individual members of the outer whorl as well, sometimes with an asymmetric result, as in *Tropaeolum*. But the trend towards

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[1] Loc. cit.

[2] No fresh specimens of this genus could be obtained for investigation, but herbarium material and published figures and descriptions certainly suggest that here, as in the closely allied *Platystemon*, the gynoecium is composed of valve carpels only, though differing from the latter type in being trimerous. A fuller knowledge of the arrangement of the vascular tissue is, however, needed for final proof.

reduction might take effect in other ways. Some carpels might diminish in bulk, and so become differentiated from the others, and thus bring about polymorphism. We may conjecture that in the first place the lamina of the valve carpel became obsolete, leaving only the midrib, or even merely its vascular cord, and that this consolidation taking place in the outer whorl may have acted as a predisposing cause of the assumption of the semi-solid form by the succeeding whorl, itself perhaps already on the way to become solid, but caused to develop in a new direction by the new conditions set up in consequence of the alteration in the outer whorl ; particularly if, notwithstanding this modification, a full complement of seed is matured, thus precluding any considerable lessening in size of the loculus. (See interpretation of *Fritillaria Meleagris*, L., *Lilium Martagon*, L., and Garden Tulip, pp. 158–65.) It would appear, however, that transformation of the valve into the semi-solid form may take place independently of such causation, seeing that in *Fritillaria Imperialis*, L., *both* whorls are composed of semi-solid carpels only ; unless, indeed, the fact that the outer three are smaller and sterile may be an indication that their present semi-solid form is the result of a secondary modification following upon the earlier change of the valve into the solid type. That a double transformation of this kind can take place appears to be established by the peculiar and instructive case of *Capsella Heegeri*, Solms., which will be considered later (see p. 139). Nor need we be surprised at this order of development (valve—solid—semi-solid), since we are familiar with many instances of mutations in which the larger change is made first, the intermediate stage not appearing until later.

In another class of case the members of the outer whorl failed to achieve the outcurve and expansion which produce the loculus. Their vascular cords remained in their central position, while those of the inner whorl were able, perhaps in consequence, to expand and produce typical valves (*Geranium, Erodium, Pelargonium*). We meet with the converse case in certain Cruciferae. The *immediate* ancestors of the present-day types of this family we may take to have had a gynoecium with an outer whorl of four orthogonal valve carpels and an inner whorl of four diagonal solid members.[1] This construction is still seen regularly in the forms known as *Tetrapoma* and *Holargidium* ; in *Capsella Vignieri*, Blar., *Brassica campestris*, var. *Sarson*, Prain, *Tropidocarpum capparideum*, Greene ; occasionally in *Lepidium sativum*, L., and rarely in a number of other genera.

As polymorphism arose, the stigma-bearing and the ovule-bearing functions often came to be performed by one kind of carpel only. But though the distribution of stigmas and placentae among the different carpel members usually became constant for the species and often for the whole

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[1] For a statement of the evidence upon which this conclusion is based, see this journal, loc. cit.

genus, in neither case did it appear to be the result of any fixed interrela-
tionship between one carpel type and another. The distribution of the two
functions has therefore to be investigated for each case.

As regards the fruits, those which became dry as they matured for the
most part dehisced longitudinally, some septicidally, some (supposedly)
loculicidally. On the accepted view, loculicidal dehiscence involves a split
down the middle of a valve carpel either through the midrib itself or to one
side of it, with the lateral veins left in connexion on the one side and
disrupted on the other. Whether loculicidal dehiscence of this nature can
take place is open to doubt. Such a mode of dehiscence, regarded purely
as a mechanical operation, would not appear to be ordinarily easy of
accomplishment. In the types so far examined the opening up of the
loculus is most frequently the result of the separation of the twin bundles
of the midrib of *semi-solid* carpels, but *Primula* is perhaps a genuine case.

With this brief reference to certain general aspects of the theory of
polymorphism, we may pass to a more detailed consideration of a number
of illustrative cases drawn from a wide range of families which go to prove
that everywhere evolution in the gynoecium has proceeded along the same
lines, and that polymorphism is met with wherever one turns.

V. Further Evidence of the Polymorphic Character of the Carpel.[1]

Dicotyledons.

Berberidaceae. *Epimedium pinnatum*, Fisch., *E. alpinum*. L., *E. viola-
ceum*. Morr. and Decne., *Vancouveria* (trimerous), *Jeffersonia diphylla*, Pers.
G 2, one a small valve, the other large and semi-solid. (Hitherto described
as G 1.) (Figs. 4-8.)

The evidence in *Epimedium* is exceptionally clear. The fruit when ripe
splits down the two sides along the border where the two systems of venation,
the one arising from the midrib of a sterile valve, the other from the central
cord of a fertile semi-solid carpel, meet but do not unite (Fig. 5). As a result
of dehiscence the sterile valve carpel, which is shorter than the other, becomes
detached, leaving behind the now opened up semi-solid fertile carpel bear-
ing the style and centrally placed placentae with two rows of ovules. The
same plan of construction may be safely assumed to occur in its near allies,
Jeffersonia (Figs. 7, 8) with incomplete transverse dehiscence, and
Plagiorhegma with its dehiscence line in an intermediate position.[2] The

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[1] For greater convenience in dealing with certain problems some families have been dealt with
out of their natural order.

[2] Doubtless also in *Croomia*, originally placed by Torrey in Berberidaceae but since transferred
to Roxburghiaceae.

view that the gynoecium in *Epimedium* is composed of two carpellary leaves was put forward by Morren and Decaisne in 1834.[1]   These authors remark upon the double system of venation, but rather curiously do not for
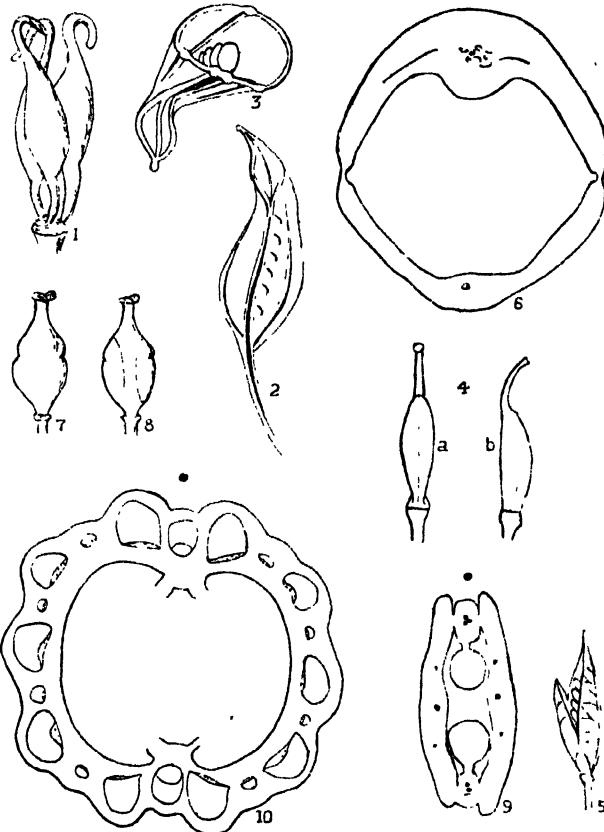
FIGS. 1–10.   1. *Coptis trifolia*, Salisb., thalamus with three follicles.   2–3. *C. asplenifolia*, Salisb.   2. A single ripe follicle showing palmate venation.   3. The same cut transversely.   4. 6. *Epimedium pinnatum*, Fisch.   5. *E. alpinum*, L.   4. Young fruit : *a*, valve view ; *b*, side view. 5. Ripe fruit already dehisced, showing the two systems of venation, one arising from the midrib of the sterile valve, the other from the midrib of the fertile semi-solid carpel.   6. The ovary in cross-section, showing the vascular bundles of the two midribs and the thin places in the wall (right and left) where the two carpels unite, and later split asunder.   7, 8. *Jeffersonia diphylla*, Pers.   7. View of fruit, showing the horizontal line along which dehiscence takes place.   8. View of same from the opposite (placental) side.   9. *Corydalis nobilis*, Pers.   Transverse section of the ovary ; the two valve carpels each show lateral veins as well as the midrib.   10. *Eschscholzia californica*, Cham. Transverse section of the ovary with twenty vascular cords : two median placental (replum), and on each side one to each of the five ribs (solid carpels) and one (valve carpel) between each rib. (Figs. 1, 7, 8, after Gray ; 2, 3, after Hooker ; 5, after Prantl ; 10, after Hildebrand ; 4, 6, 9, original.)

some reason represent the ovary of *E. alpinum* as two-membered in the diagram (Table 12, Fig. B).   Further, although describing both carpels as being of the same nature, they yet make no reference to the difficulty

[1] Ann. sci. nat. Bot., 2ᵉ sér., ii, p. 358.

inherent in such a construction in the case of valve carpels owing to the position of the ovules. Put forward in this form, indeed, this interpretation could only commend itself to those holding the view that ovules are axial appendages and are not borne by the carpels. Baillon, however, dissents from the French standpoint and dismisses the two-carpel idea as untenable.[1] But one may more justly contend that what is inexplicable is how an ovary, if it be in fact composed of a single carpel of the orthodox valve type, could have the following characters: (1) a midrib which extends only about half-way up the ovary wall, (2) a mode of dehiscence which separates this midrib with a strip of tissue on either side from the remainder of the carpellary leaf, (3) a double system of venation, (4) a style composed of the edges of the carpel lamina in which the midrib takes no part. On the polymorphic view these difficulties disappear. Moreover, the organo-genetic evidence that two protuberances are seen in the earliest stage of development is further proof, if it were needed, that G = 2.

Papaveraceae (including Fumariaceae). G 4–∞, rarely 3; carpels almost always alternately valve and solid,[2] rarely all valve or all solid. (Hitherto described as G 2–∞.) (Figs. 9–17.)

As this family has been discussed in the earlier account, little need be said here. The minimum number of carpels is apparently three (*Platy-stigma*, Benth.).[3] In this genus and in *Platystemon*, where the styles and stigmas stand over the valves, the gynoecium appears to be composed entirely of valve carpels, but in other genera both valve and solid carpels are present, of which the latter alone as a rule bear the (so-called commis-sural) stigmas and ovules. In the genera with siliquiform fruits only the median pair of carpels forming the replum frame is normally fertile (Figs. 9, 10), but individual fruits have been observed in *Eschscholzia* and *Hunnemannia* in which a neighbouring solid carpel also participates in the ovuliferous function.[4] In the three closely related, many-carpelled genera *Eschscholzia, Dendromecon,* and *Hunnemannia* we meet with the *compound* valve, a structure simulating a single typical valve carpel but composed of numerous sterile solid and valve members which become detached together as one whole. The case of *Eschscholzia*, which has the styles distinct, is of particular interest, for in these structures an atavistic series comparable with the phenomena observed in *Matthiola* and many other Cruciferae[5] is of constant occurrence (Figs. 11–16). It would seem probable

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[1] A pronouncement which it must be remembered, however, takes cognizance of only one kind of carpel (see Hist. Pl., iii, p. 55, foot-note 3).

[2] In this arrangement we find a clue to the characteristic mode of dehiscence of the fruit by pores, a result due simply to the separation of the valve carpel at its apex from the solid carpel.

[3] See foot-note 2, p. 129.

[4] Such an occurrence, though considered probable, had not actually been established in the earlier account.

[5] See foot-note, p. 130.

that in the ancestral *Eschscholzia* type all twenty carpels[1] must have normally produced styles. To-day the usual number during the first part of the season is four, dwindling to two later. But among the earliest flowers it is not rare to find as many as eight, twelve, and even sixteen style
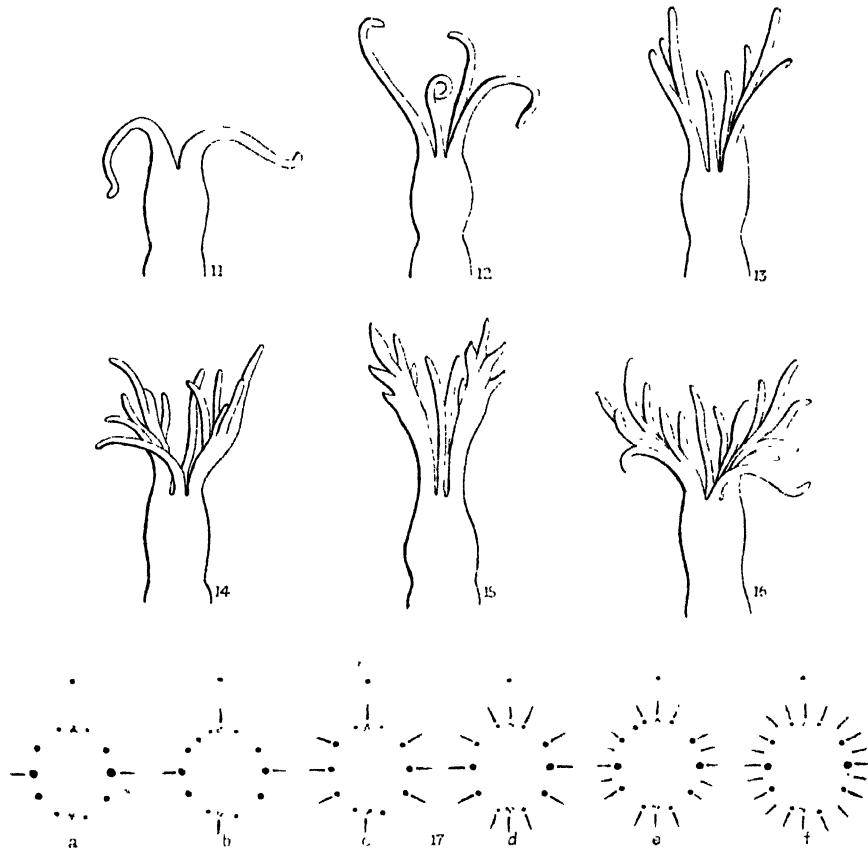
FIGS. 11–17. *Eschscholzia californica*, Cham. (11–16 semi-diagrammatic.) Apex of the ovary showing 2 (Fig. 11), 4 (Fig. 12), 8 (Fig. 13), 12 more or less free (Fig. 14), 12 more or less joined (Fig. 15), 16 (Fig. 16) styles and stigmas. 17, *a-e*. Diagram showing the position of the styles according as the number present is 2, 4, 8, 12, or 16. *f*. Ground plan (theoretical) of an ovary in which every carpel produces a style.

structures, and I have no doubt that as many as twenty might very occasionally be traceable. The order of disappearance is quite regular, and is as follows: the styles are lost first from some of the eight-valve carpels (as is to be observed in the case where sixteen remain, see Fig. 16); then

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[1] That is to say, two median replum carpels together with five solid and four intervening valves forming each compound valve. There is no valve carpel separating the replum carpels from their solid neighbours (see Fig. 10).

from the whole number of valve carpels, leaving only twelve (i. e. those belonging to the ten sterile and two fertile solid carpels, see Figs. 14 and 15); then from the outer solid members of each lateral group of five, thus leaving eight (Fig. 13). Next follow the now outer members of the two lateral groups of three, in this way producing the typical four-styled condition with two lateral and two median members (Fig. 12). Finally the replum styles also disappear, and only the central style of each compound valve remains (Fig. 11). The sequence is shown diagrammatically in Fig. 17 (proceeding from right to left).

Cruciferae. G 4–8–∞ , carpels. commonly dimorphic, large valve and solid in the siliqua, small valve and semi-solid in the silicula. (Hitherto held to be G 2, with rare variations in individual fruits to G 3 or G 4.) (Figs. 18–35.)

Here, too, a brief statement will suffice, since both evidence and argument have been set out at length in the earlier account. In the majority of genera in which the fruit is a siliqua we find two median solid and two lateral valve carpels present. reduction having in these cases apparently reached a point of stability which may well represent the limit of the process in general, though it may be that the one-sided development seen in the later-formed [1] fruits of *Schimpera persica*, Boiss., and throughout in *Schimpera arabica*, Hochst. and Steud., where one loculus only is fertile, marks the first step in the direction of suppression of yet another (valve) carpel member. The solid carpels are almost invariably fertile, and in most cases bear the (commissural) stigmas, while the valve carpels are nearly always sterile and generally destitute of stigmas. The converse relations are illustrated in the exceptional cases of *Biscutella* (solid carpels sterile, valve carpels fertile and contributing to the formation of style and stigma) and *Matthiola* (stigmas centred over the valves). In passing it may be noted that in *Biscutella* fruits sometimes occur in which one valve is completely lacking. In these cases the replum and style are nevertheless similar in form to those of normal fruits—yet one more proof that the replum cannot be formed of the margins of the two lateral carpels, since here one alone is present. In genera with carpels more than four the number is often indicated by various external features, as e. g. prominent ribs, ridges, or wing-like outgrowths. In the many-carpelled forms in which the fruit is ribbed, jointed, and indehiscent, as in *Rapistrum* and *Enarthrocarpus*, we have the counterpart of the *Hypecoum* type among the Papaveraceae-Fumariaceae. In all three genera consolidation has been carried to the maximum without corresponding reduction in number. In this class of fruits, in addition to the central loculus, there may be present a ring of subsidiary loculi around or between the seeds, related in number to the number of carpels. Many such all-solid-carpelled fruits have the lowest

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[1] The earliest fruits are symmetrical, straight, and with a shorter beak.
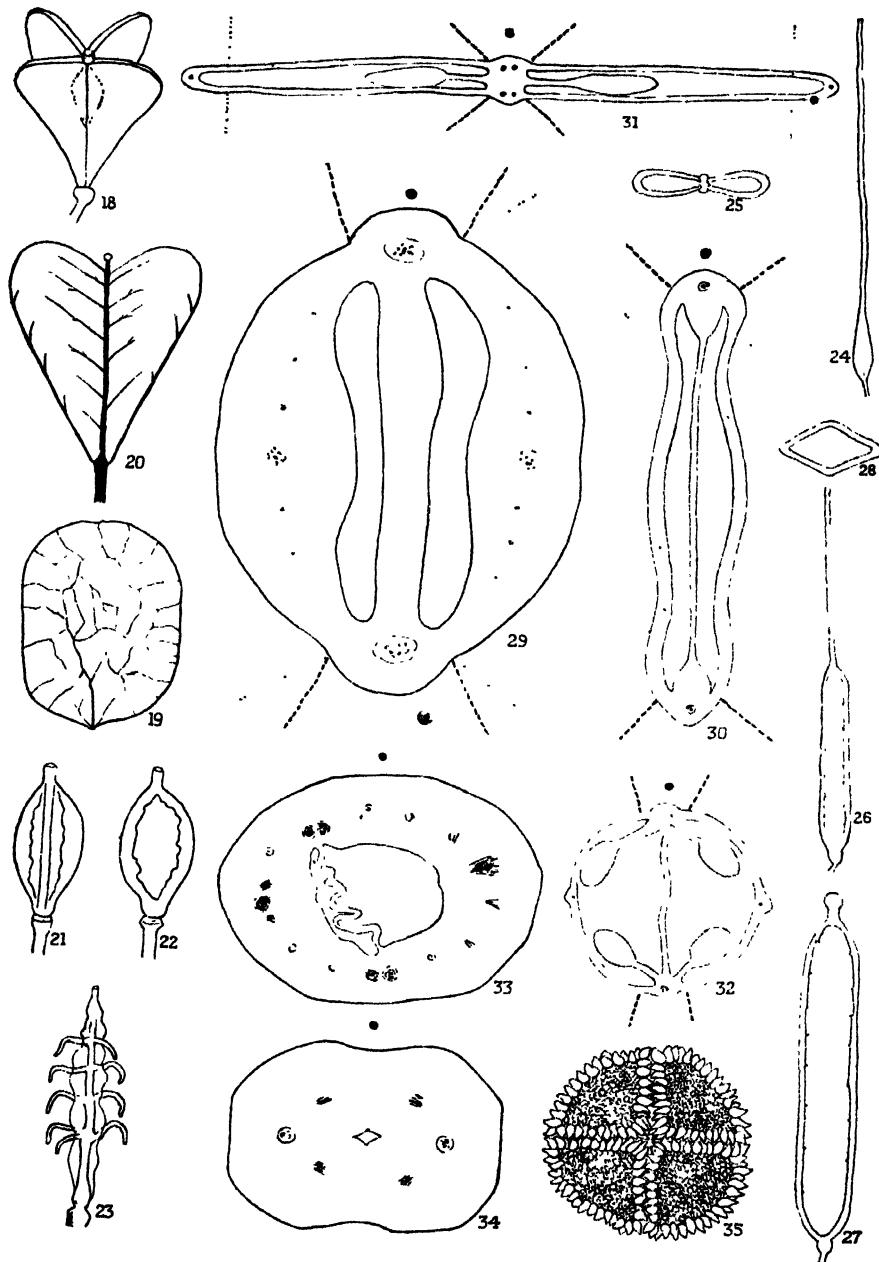
FIGS. 18-35.   18. *Capsella Viguieri*, Blar., fruit.   19. *Lunaria annua*, L.   One of the fruit valves, showing the main veins which are derived from three different carpels (see text).   20. *Capsella Bursa-pastoris*, Moench. Fruit injected with eosin solution, showing the venation systems arising from the two solid carpels (right and left) and from one semi-solid carpel (centre).   21-3. *Capsella*

joint sterile. In the present state of our knowledge we can but speculate as to whether in these types the ovules farthest from the stigma failed frequently to be reached by a pollen-tube and whether their constant atrophy led to a non-expandable state of solidification in the bottom segment. Or whether the condition making for consolidation existed in fullest measure at the moment of origin, but, having found expression in the basal region of the ovary, declined as development proceeded, an interpretation which finds a certain parallel in the frequent outburst of atavism (three- to four-valved condition) in the *lowest* flowers on the axis, after which the impetus seems to become exhausted, unless development is later interrupted by some check and then again resumed, in which case the reversionary condition may reappear. Persistence of the four-valved condition in succeeding (one or more) generations has been recorded for *Tetrapoma barbaraefolia*, Turcz., *Holargidium Kusnetzowii*, Turcz., *Brassica campestris*, var. *Sarson*, Prain, *Capsella Viguieri*, Blar. (Fig. 18), *Lepidium sativum*, L., and must also evidently occur in *Tropidocarpum capparideum*, Greene. In all the above forms, however, the ancestral character is found generally, if not invariably, to disappear in the fruits formed at the end of the season, these being of the normal two-valved pattern. Furthermore, it is acknowledged that none of these forms are distinguishable from the species which they resemble[1] in any other respect than in the fruit character. This is fully comprehensible now that we can regard them, not as distinct species, but merely as atavists capable of remaining stable under a wider or narrower environmental range. Of the above-mentioned genera *Tropidocarpum* is of special interest in that individuals are met with bearing several different kinds of fruits,[2] some having reverted fully (four-valved), some partially (three-valved), some showing a curious transition some way up the siliqua, being rhombic in cross-

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Heegeri, Solms. 21. Suture view. 22. Valve view of the fruit. 23. One of the solid (replum) carpels with funicles attached. 24, 25. *Tropidocarpum gracile*. Hook. 24. Replum and septum. 25. Ovary in cross-section. 26. Replum and partial septum of the '*dubium*' form. 27. Replum of '*rhombic*' form. 28. Ovary of '*rhombic*' form in cross-section. 29–32. Ground plan of the ovary of different Crucifer types, showing the points of dehiscence (heavy-dotted lines) and junctions of the carpels (light-dotted lines). 29. Typical siliqua. 30. Latiseptal silicula of *Lunaria annua*, L. 31. Angustiseptal silicula of *Capsella Bursa-pastoris*. Moench. 32. *C. Heegeri*, Solms. 33–5. *Crambe maritima*, L. 33. Transverse section of the ovary in the ovuliferous region, showing the midribs of the four carpels, those of the median pair with twin vascular bundles; the replum is pushed to one side by the developing ovule (not shown). 34. The same at a higher level; the double bundles of the median carpels have now diverged considerably. 35. Transverse section of the same in the region of the stigma, showing four wedge-shaped segments fringed with the stigmatic papillae. (Fig. 18 after Blaringhem; 21, 22, after Solms-Laubach; 24–8, after Robinson; the rest original.)

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[1] Viz. *Nasturtium palustre*, DC., *Draba* (? *hirta*, L.), *Brassica campestris*, L., *Capsella Bursa-pastoris*, Moench., *Lepidium sativum*, L., *Tropidocarpum gracile*, Hook. *Capsella Viguieri*, it is true, is described by Blaringhem as a fasciated plant, but this latter condition we may suppose to be incidental to, rather than inherent in, the atavistic character.

[2] See B. L. Robinson in *Erythea*, vol. iv, p. 109 and Plate 3.

section and unilocular in the lower half, very much flattened from back to front, and bilocular above (*dubium* form), while some are strictly two-valved and flattened laterally throughout (*gracile* form).   The evidence all points to the conclusion that *T. capparideum* and *T. dubium* are merely relatively stable and unstable[1] atavistic forms respectively of *T. gracile*, Hook.   The full significance of the change of form occurring some way up the siliqua of *T. dubium* will be more easily made clear after we have dealt with the case of *Capsella*.

Up to the present attention has been directed mainly to the siliqua-forming types or to types whose fruits, though dry, are indehiscent; further consideration must now be given to those forms in which the fruit is a silicula.   Of these the dehiscent section is commonly subdivided into the *latiseptae* with a broad, and the *angustiseptae* with a narrow dissepiment. This mode of describing the two groups, though convenient and, as far as it goes, true, leaves us unenlightened as to the underlying nature of the distinction, which rests upon the different character of the carpels.   If we take as our examples the two familiar plants Honesty (*Lunaria annua*, L.) and Shepherd's Purse (*Capsella Bursa-pastoris*, Moench.) and observe the venation of the fruit, we see at once that the median carpels in both cases are not solid as they are in the siliqua, but semi-solid with wide lateral expansions, while the lateral carpel pair have undergone a corresponding reduction. · In *Lunaria* (latiseptal) the fruit is extended in the median plane, consequently the replum is broad from back to front, and the sides of the fruit, which are closely approximated to, and parallel with the replum, are also broad.   At maturity these sides become detached from the replum like the true valves in the siliqua, but unlike these latter structures they do not each represent an individual lateral carpel.   The lateral carpels here occupy only a small undefined region of the large oval valve face.   The midrib is seen as a small vein which runs up the centre of this face only for a very short distance[2] before breaking up into reticulations which connect with the ultimate ramifications of the median semi-solid carpels (Fig. 19). These latter members are folded so that the two halves of the lamina face each other, the cross-section being V-shaped.   Their midribs run along the edges of the fruit, and thence pass up into the style, which ends in a bifid stigma.   The contrast in size between the two pairs of carpels is fore-shadowed in the slender stalk-like base composed of the united carpel petioles, where the four bundles for the midribs are already separate, the two for the lateral pair appearing as very slender threadlike strands, while those for the median pair form two massive cords.   When the fruit is ripe

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[1] In the sense that the condition is not manifested throughout the siliqua.

[2] In this respect the two valves are often dissimilar, the midrib bundle being frequently quite apparent in the one but barely traceable in the other, an inequality which need not surprise us in the case of paired structures which have undergone considerable reduction.

the primary lateral veins of the semi-solid carpels snap from their midribs precisely in the manner of certain of the Papilionatae (see later, p. 146), and the two *fruit* valves fall. One such valve thus represents one much reduced lateral carpel together with one half of each of two semi-solid median carpels. The same process occurs in *Capsella,* but in this case the fruit is flattened from back to front, i. e. in the plane at right angles to the plane of flattening in *Lunaria,* and the shape relations of the carpel pairs are reversed. Here it is the median semi-solid members which are flat and the very reduced lateral carpels which have the lamina folded, the midrib, as in *Lunaria,* running along the fruit edge. All four midribs enter the style in *Capsella,* which has a simple capitate stigma. The reason why no sutural contour lines appear on the surface of the silicula such as always characterize the siliqua is now obvious. The double contour line in the siliqua marks the junction of the solid carpel with its valve neighbour on either side. In the silicula the wings of the semi-solid carpels are not delimited from the valve carpel edges, the one passing imperceptibly into the other. The two different venation systems of the *Capsella* fruit are very clearly seen, the one springing from the midribs of the semi-solid pair with many strong lateral ribs, the other from the midribs of the reduced valve members which give rise to but few lateral veins. The two systems become continuous through their ultimate reticulations, but if a cut shoot be placed in an aqueous solution of eosin the injection process can be stopped at the stage at which the two sets of midribs and primary laterals, but not the communicating network, are outlined in colour, thus rendering beautifully distinct the main vascular system proper to each carpel (Fig. 20).

The construction of the fruit of the type species and of *C. Viguieri,* Blar., satisfactorily interpreted, it remains to consider the peculiar and interesting case of *C. Heegeri,* Solms. Descendants of the two original plants discovered by Heeger have been fully described by Solms-Laubach, but owing to the fact that in his figures the vascular bundles are not represented (Figs. 21 and 22), I was for some time unable to come to a decided view as to the precise nature of the modification exhibited by the fruit in this rare form. Attempts to procure the living plant were unsuccessful, but eventually two individuals were raised from seed kindly sent to me by M. Meunissier Of these two plants—the only ones obtained from a sowing made late in the season [1]—one proved to be genuine *Heegeri* and the other intermediate[2] between *Heegeri* and the type. Examination of this material at once removed all doubts. Evidently the median carpels have here contracted from the typical expanded semi-solid almost to the narrow solid shape, while the replum development is largely increased, so that the whole back

[1] From a later spring sowing a large number of individuals were raised, some being true *Heegeri* but most of an intermediate form, either throughout or showing an occasional fruit of true *Heegeri*.

[2] In the intermediate form the upper edge of the fruit valves neither slopes upwards as in *Heegeri* nor downwards as in *Bursa-pastoris,* but runs horizontally.

to front dimension is now considerable instead of being reduced to a mini-
mum, as it is in *Bursa-pastoris*; the mesophyll tissue is also increased and
the funicles are longer (Fig. 23). Coincidently with this change, the lateral
valve carpels form a more extended arc, having now the normal valve
shape instead of being conduplicate, so that the ovary becomes nearly
circular in cross-section (Fig. 32). Is this a case of the siliqua plan of con-
struction re-emerging in a form in which it originally existed, before
modification to the angustiseptal silicula shape occurred with accompanying
loss of capacity for extended growth in length, a capacity which is not
reacquired when reversion to the original ground plan comes about ? That
it may be so is all that with our present knowledge we can venture to say.
But we have gained from this study a hint as to a possible explanation of
the apparently unaccountable change in configuration which occurs some
way up the siliqua in *Tropidocarpum dubium* (see above, p. 137). It seems
possible[1] that the condition which characterizes the fruit of *Capsella
Heegeri* and in this plant affects the whole silicula, in *Tropidocarpum* is
sharply localized in the lower half of the siliqua, so that we get a sudden
transition from what we might term the *Heegeri* condition to the normal
taking place in the course of the length of the siliqua. (A further complica-
tion may arise owing to a certain degree of torsion of the fruit, such as is
met with in some Papaveraceae, as e. g. *Glaucium*,[2] but this is a phenomenon
distinct from the change in carpel form.) Finally, we may surmise that in
the form distinguished as ' rhombic ' we have an exact parallel to *C. Heegeri*,
the condition which in *dubium* exists in the lower part of the siliqua only
being here maintained to the top. When it is added that all three forms
of ground plan (*gracile, dubium,* ' rhombic ') together with both three- and
four-valved fruits have been found on a single individual, the amazing
plasticity of the carpel relations in this genus will be realized. The corre-
spondence between the whole *Tropidocarpum* series, as here interpreted, and
those of *Capsella* is exhibited in tabular form below :

Tropidocarpum. Fruit a siliqua.	*Capsella.* Fruit a silicula.
T. gracile. Siliqua of exceptional (? unique) construction, the fruit valves being conduplicate instead of flattened.	*C. Bursa-pastoris.*
' rhombic form.'	*C. Heegeri.*
dubium form (half ' rhombic ' half *gracile*).	Some intermediate fruits of *C. Heegeri* approach the condition of being *Heegeri* at the base, and *Bursa-pastoris* in the upper part.
capparideum form (G 4 + 4).	*C. Viguieri* (G 4 + 4).

We thus arrive at the conclusion that in the Cruciferae evolution in the
gynoecium has proceeded along two different lines as the result of carpel

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[1] Unfortunately no material of *Tropidocarpum dubium* could be obtained which would permit of
this suggested explanation being verified.

[2] Pflanzenfamilien, iii, 2, p. 135, Fig. 83 c.

polymorphism and of the occurrence of different combinations of the various carpel types. When the median carpels become solid the characteristic form is a siliqua, when they become semi-solid the result is a silicula.[1] In these circumstances it is not a little remarkable that the planes in which the rupture of the tissues takes place in the ripe fruit should be identical for the siliqua and both types of silicula. The orientation of the mechanical tissue concerned in dehiscence appears to be fixed and to be independent of the position of the carpel boundaries. The accompanying diagrams will serve to illustrate this point. It will be seen that in the typical siliqua, in which there are but few vascular connexions between valves and replum, the line of dehiscence coincides with the line of junction of two different carpel types (Fig. 29), while in the silicula it cuts clean across the central region of the median pair, the numerous lateral veins snapping across at their point of origin on both sides of the midrib (Figs. 30 and 31). In the exceptional case of *Capsella Heegeri*, however, where the lateral carpels are of the valve type, junction and dehiscence lines coincide as in the siliqua, though owing to slight expansion of the median carpels they are situated at a little distance from the midrib (as shown in Fig. 32). This may account for the fact that in this form the valves are detached with some difficulty. This disposition of the forces causing dehiscence independently of those which determine the line of division between the carpels is not unlike the phenomenon observed by W. and A. Bateson in *Veronica*, where the forces governing the division of the corolla into petals were found to be disposed in different ways, while the orientation of the colour scheme remained constant.[2]

When the fruit is succulent the outward distinction between the two pairs of carpels is often slight, and the determination of the category to which they should be referred less easy. In *Crambe maritima*, L., which may be taken as illustrative of this class, a cross-section of the basal sterile joint shows the two pairs of midribs, and in the upper expanded region the single bundle of the two sterile lateral carpels and the twin bundles of the fertile median pair (Fig. 33). At the apex these twin bundles diverge considerably, so as to lie almost alongside the lateral midribs (Fig. 34). This behaviour explains the form of the stigmatic disc when, as is sometimes the case, it is marked off by median and lateral depressions into quadrants and in transverse section shows four wedge-shaped segments fringed with the stigmatic hairs, corresponding in their orientation with the

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[1] The only alternative to this interpretation, since the evidence for the occurrence of four carpels appears to be irrefutable, would seem to be one which concedes the presence of four carpel midribs, but which regards the *lateral* venation system as a secondary adaptation without significance in this connexion. But there is no evidence that this is the case, and such an explanation would remove none of our present difficulties.

[2] On Variations in the Floral Symmetry of Certain Plants having Irregular Corollas. Journ. Linn. Soc., Botany, vol. xxviii, p. 386, 1891.

position of the endings of the four bundles derived from the two fertile carpels (Fig. 35).

Caryophyllaceae.   G 2–3–5 + 2–3–5.   (Hitherto considered to be G 2–3–5.) (Figs. 36, 37.)

Dianthus sp.   G 2 + 2.   A transverse section taken through the base of the gynoecium shows two main vascular cords in the median plane, which, bending outwards, form the midribs of two median valve carpels enclosing the two loculi (Fig. 36, *a*). Immediately above this level, two more vascular cords run out horizontally in laterally placed septa to the ovary wall, forming the midribs of two solid carpels. The growth of the parenchymatous tissue intervening between the midribs of these latter carpels and their placentiferous bundles which are left in the centre is unable to keep pace with the enlargement of the whole ovary, and the septa rupture, the torn edges being recognizable up the whole height of the ovary cavity (Fig. 36, *b*).

Lychnis vespertina, Sibth.   G 5 + 5.   Here we meet with a similar construction in a pentamerous type.   The disposition of the vascular cords and the manner in which those of the solid carpels are gradually used up in furnishing branches to the funicles are shown in van Tieghem's drawings.[1]

Leguminosae.   G mostly 2, sometimes 10–12 or more, carpels semi-solid or solid. (Hitherto described as G 1 in all but a few exceptional genera among the Mimoseae and Caesalpinieae.)   (Figs. 39–49.)

The legume, the typical fruit of the three sub-families of the Leguminosae (Mimoseae, Caesalpinieae, Papilionatae), has always been regarded as one of the most characteristic examples of a one-carpelled ovary.   There are, however, a number of facts which this interpretation will not cover. Moreover, the whole weight of evidence derived from the disposition of the vascular system is against this view, and shows that the pod is undoubtedly a two-carpelled structure.   This is perhaps most clearly demonstrated in *Haematoxylon campechianum*, L. (Caesalpinieae), where we have exhibited the (? unique) case of a pod dehiscing down the middle of each flat side instead of along the two sutures.   Here the two carpels are both semi-solid and of nearly equal size, the ovules being borne on placentae occupying the normal position.   These placentae do not, however, as hitherto conceived, represent a real suture, but are situated on either side of the vascular cord constituting the midrib of one carpel (vexillary), a similar and separate cord passing up the opposite edge of the pod to form the midrib of the other (carinal [2]) carpel, which is sterile.   Two distinct systems of venation spring from the two midribs and tail off towards the centre of each flat side, thus

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[1] Recherches sur la Structure du Pistil, Pl. XI, Figs. 323–35.

[2] The terms 'carinal' and 'vexillary' are employed in order to avoid ambiguity, the more usual expressions 'dorsal' and 'ventral', 'anterior' and 'posterior' having been used by different writers in opposite senses.

rendering possible a mode of dehiscence inconceivable in a one-carpelled fruit. Such sub-equal development of the two members of the gynoecium is, however, rare. In most leguminous genera the sides of the pod are composed *entirely* of the lamina of the *fertile* member, and this preponderating development of the one carpel is counterbalanced by the reduction of the other to the solid column containing the vascular cord. This can be very clearly seen with the naked eye in the bladder-like pods of *Colutea* (Papilionatae). From the twin bundles of the (so-called) ventral suture, actually
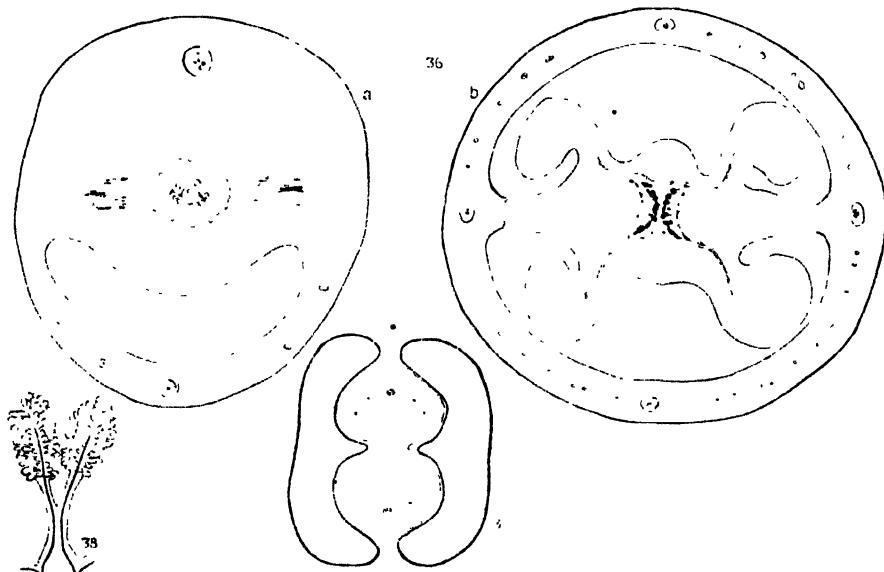
FIGS. 36–8. 36, 37. *Dianthus* sp. 36, *a*. A section through the base of the ovary slightly oblique); the posterior member of the lower pair of (median valve) carpels still coalescent with the axis; the anterior member has curved outwards to form the loculus; the vascular cords of the second (lateral, solid) pair forming the septa are running out horizontally in order to complete the ovary wall. 36, *b*. Transverse section of the same at a higher level, showing the ruptured septa; the ovule on each side of a septum belongs to the same carpel, as indicated by the distribution of the placental vascular bundles in the centre (on the old view of G 2 they should belong to different carpels). 37. Transverse section through the summit of the ovary, showing the formation of the two styles from the median carpels and the arcs of horny tissue which form the four valve-like teeth in the ripe, split capsule. 38. *Drosera rotundifolia*, L., showing that the apparently 2-split stigma really consists of two half-stigmas from two neighbouring semi-solid carpels whose vascular cords bifurcate (see p. 151). (Fig. 38 after Drude; the rest original.)

the midrib of the fertile vexillary carpel, spring strong lateral veins right and left in a symmetrical manner. These primary lateral veins break up into finer reticulations as they extend round the circumference of the pod, the terminals finally abutting on, and often for a short distance lying alongside of, the midrib of the solid carpel on the opposite (anterior) side (Figs. 39 and 40). To account for this regular herring-bone pattern of lateral branches springing from the vexillary cord and for the direction in which they fork, on the current view that G = 1 with the suture vexillary, is to

accept that the ordinary mode of development of the foliar vascular system can be reversed, and that in a carpel the midrib can give rise directly to a fine reticulation which gradually gathers up into a small number of large veins, which finally become united to a common and a still larger (marginal) vein running at right angles to these last-named veins, an interpretation which, in this case, it hardly seems possible to uphold. *Medicago* affords another instance in which proof of the presence of the two carpels is plain to the eye. In certain smooth-fruited types such as *M. orbicularis*, All., the venation of the several spires of the ripe fruit stands out in strong relief, and shows a but slightly branched system radiating from the double vascular cord edging the concave border, i. e. from the midrib of the fertile carpel, and reaching to, or but little short of, the outer margin (Fig. 41). As the fruit becomes fully ripe the other vascular cord along this outer edge (the juxta-marginal vein of systematists) separates for the most part cleanly from the spires like a tyre from a wheel, leaving visible the now free endings of the laterals of the other midrib, many of which show the L-shaped (with the short arm pointing sometimes up, sometimes down) or T-shaped terminations by which they were applied without actually becoming united to this marginal vein (Fig. 42). Here we see again the great predominance, almost universal among the Papilionatae, of the semi-solid fertile member over the sterile carpel, which becomes reduced to a vascular cord. But we meet with a different type of construction in the many-ribbed indehiscent fruits of *Arachis* and *Scorpiurus*. Here we have exemplified the condition of ' consolidation without reduction ' with which we have already become familiar in the Rhoeadales in such types as *Hypecoum* and *Rapistrum*, but which is rare among the Leguminosae. In *Arachis hypogaea*, L. (monkeynut), the fruit has from ten to twelve parallel and equally strong fibrovascular cords running from base to apex, representing a corresponding number of solid carpels whose reticulations unite to form the intervening network (Figs. 43–5). The median vexillary cord shows in its course two or three nodal points connecting with the neighbouring cord on either side (Fig. 44) (best seen after the papery skin has been carefully removed) to which the funicles are attached. Otherwise all vascular cords appear alike and pass separately down into the pedicel. In *Scorpiurus* (Figs. 46 and 47) the carpel number seems to range between ten and fourteen, all being solid except the median carinal member, which forms almost the whole of the concave face of the spiral and which with its distinct pinnate venation may be considered to belong to the valve type. The ovuliferous carpel which occupies the mid-position on the convex (vexillary) side is reduced to its vascular strand, which is somewhat sunk and lies between and is sometimes barely separated from the adjacent stronger cord on either side.[1]

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[1] Thus resembling in its position and relations with its neighbours one of the fertile (replum) carpels of *Eschscholzia*.
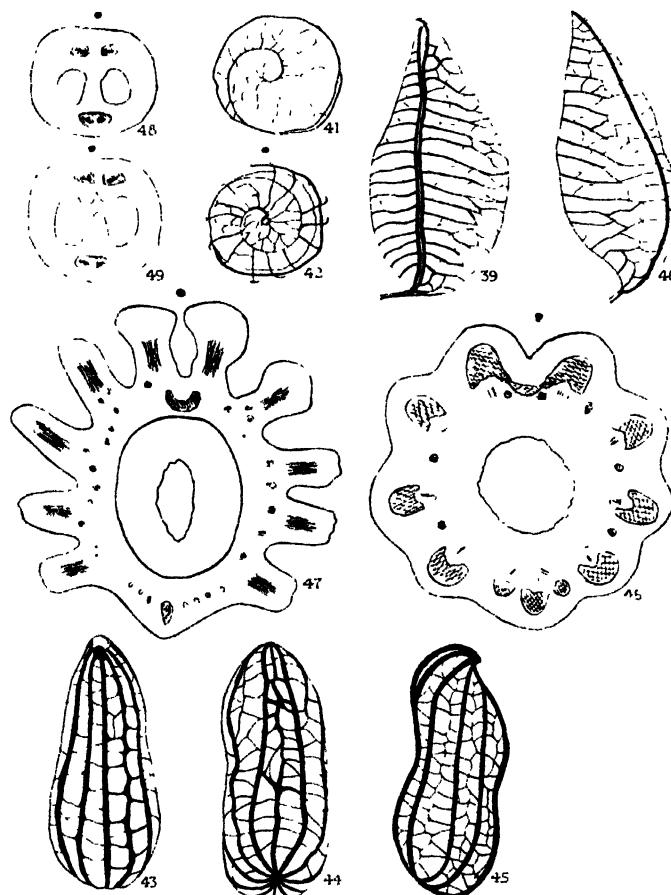
FIGS. 39 49. 39, 40. *Colutea arborescens*, L. 39. Bladder-like fruit showing the well-developed venation system originating from the vexillary (so-called sutural) vascular cord (= midrib of fertile carpel). 40. The same showing the less-developed system arising from the carinal vascular cord (= midrib of sterile carpel). 41, 42. *Medicago orbicularis*, All. Spirally coiled fruit showing the whole venation system springing from the vascular cord bordering the concave margin (= midrib of the fertile vexillary carpel) ; 41, before dehiscence, viewed from above ; 42, after dehiscence, viewed from below. 43–5. *Arachis hypogaea*, L. Fruit after removal of the papery skin, showing the vascular cords (midribs) of several of the numerous (10–12) solid carpels ; 43, viewed from the front ; 44, from the back ; 45, from the side. In 44 the central carpel shows two nodal points indicating the point of attachment of the ovules. 46. *Scorpiurus subvillosa*, L. Transverse section of an ovary of ten carpels (one with twin bundles, median, fertile, at the back, one median valve in front and four solid on each side). 47. *S. vermiculata*, L. The same, but with five solid carpels on each side, making with the two median carpels a total of 12. (In Fig. 46 the large shaded areas represent caps of sclerenchyma over the midrib bundles, which are indicated by dots. Two strong lateral veins of the anterior carpel also have caps, but these are absent from the smaller lateral veins seen here and there. The cap of the posterior carpel, which is sunk, is continuous with its neighbour on either side. In Fig. 47 the midribs of the sterile carpels run up into the processes and are seen cut obliquely.) 48,49. *Astragalus hypoglottis*, L. 48. Transverse section of the base of the ovary ; the two semi-solid carpels have not yet drawn apart in the centre. 49. The same at a higher level, showing the persistent incomplete partition formed from the central tissue of the front carpel.

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These two median members do not produce the spines or tubercles which arise in parallel rows on some (*S. sulcata*, L.) or all (*S. vermiculata*, L. (Fig 47), and *S. subvillosa*, L. (Fig. 46)) of the remaining carpels. Outgrowths of various kinds are, as has been already remarked, a common feature of sterile carpels, and particularly so, it would seem, in those of solid type.

A few other exceptional genera call for further remark. In *Carmichaelia* (Papilionatae) we find a mode of dehiscence as exceptional in its way as that of *Haematoxylon*, which it seems further to resemble in the nearly equal development of the two carpels. The walls of the pod are thick and leathery, the veins arising from the two stout midribs few in number, short and fine. When the pod is ripe these laterals are easily snapped, and the two flat sides fall out of the frame made by the two midribs, which is left behind. *Schotia* seems to behave in a somewhat similar way among the Caesalpinieae, and *Lysiloma*, *Schrankia*, and species of *Mimosa* among the Mimoseae. In *Astragalus* (Figs. 48 and 49) and species of *Oxytropis* an incomplete median partition is present which generally protrudes from the carinal side, rarely from both in *Astragalus*, and from the opposite, rarely from the same, side in *Oxytropis*. When carinal the partition is comparable with the half-replum formed from the anterior solid carpel in a Crucifer, and, as in the Cruciferae, shows in *Astragalus* a very variable degree of development from species to species. The partition is often described as double, but it is in reality single, only giving a misleading appearance of duality at a late stage through rupture of the parenchyma filling the space between the two sheets of tough parchment-like tissue underlying the epidermis on either side. The vexillary partition is a similar development from the midrib of the semi-solid fertile carpel.

It is thus apparent that a study of the venation system is essential to a clear understanding of the structure and mode of dehiscence of the legume. The typical two-carpelled pod, having one solid and one semi-solid member, splits readily along the midrib of the ovuliferous carpel and less easily down the mid-line of the sterile member, because in the former case the twin bundles supplying the two placentae generally run somewhat apart, whereas in the latter they usually form a single strand. When the sterile carpel is reduced to a mere vascular cord, the reticulations of the sides of the pod are in most cases derived entirely from the semi-solid fertile member. When both members are semi-solid the two systems may be connected by their ramifications or each may end blindly. This double system of venation is often particularly well seen in cases where the carpels are broadly winged. Where many solid carpels are present longitudinal dehiscence becomes impossible, as appears to be the case in whatever family the condition occurs.

Geraniaceae. G 5 solid + 5 valve, in proper alternation with the staminal whorls. (Hitherto described as composed of a single whorl of five carpels disposed around a prolongation of the floral axis (carpophore) and on the same radius as the petals and the inner whorl of stamens, an arrangement which transgresses the law of alternation of successive whorls.) (Figs. 50-8.)

A transverse section of the ovary of *Pelargonium* (Fig. 50), *Erodium*, and *Geranium* shows five valve carpels each with its midrib, and at the inner angle of each loculus a vascular bundle which supplies the placentae. Separating the five loculi are five broad septa, each also with a well-marked bundle (or sometimes two, one in front of the other) lying a little farther out than the alternate placental bundles, the whole ten surrounding a central pith. The outline of the section shows the larger part of the circumference to be occupied by the five valves forming the flat or convex sides, and between them five sectors which on the current view are held to be radial lamellae arising from a central prolongation of the floral axis. But if these lamellae are really axial in nature, they should, in accordance with the ' leaf-skin ' conception of the stem, be clothed with the downward extension of one or more foliar members exserted at some higher level—an impossibility, since no such members exist. But in fact these sectors are not axial in character; they undoubtedly represent an *outer* whorl of five solid carpels. As the flower developed, they failed, however, to achieve the normal outward bulging which results in a loculus, their vascular cords continuing instead the line of their upward course. Not so, however, with the inner whorl. They ' ballooned' successfully, and in so doing pushed to the exterior the members of the inner whorl of stamens, so that they came to stand farther out than the true outer whorl. which had not been forced peripherally in this manner. We thus obtain a logical explanation of the phenomenon of obdiplostemony, hitherto unaccounted for though recognized as a characteristic feature in many families. The presence in some septa of two bundles in line suggests that in these solid members the preparatory step of separation of midrib and placental bundles can be brought about, though development goes no farther. There is no running horizontally outwards of the outer midrib bundle and no expansion of the tissue on its flanks, which is essential for the formation of a loculus. Successive transverse sections in the region where the (so-called) carpophore passes into the single style show that the placental bundles of the valve carpels here come to an end (Fig. 51). Their midribs are, however, prolonged and supply the five stigmas which stand over them. The twin bundles of the (at this level) single cord in each semi-solid carpel diverge, and apply themselves to the flanks of the neighbouring valve midrib on either side for a longer or shorter distance. The method of separation of the carpels at maturity differs in the different genera. In *Erodium* the valve carpels are detached

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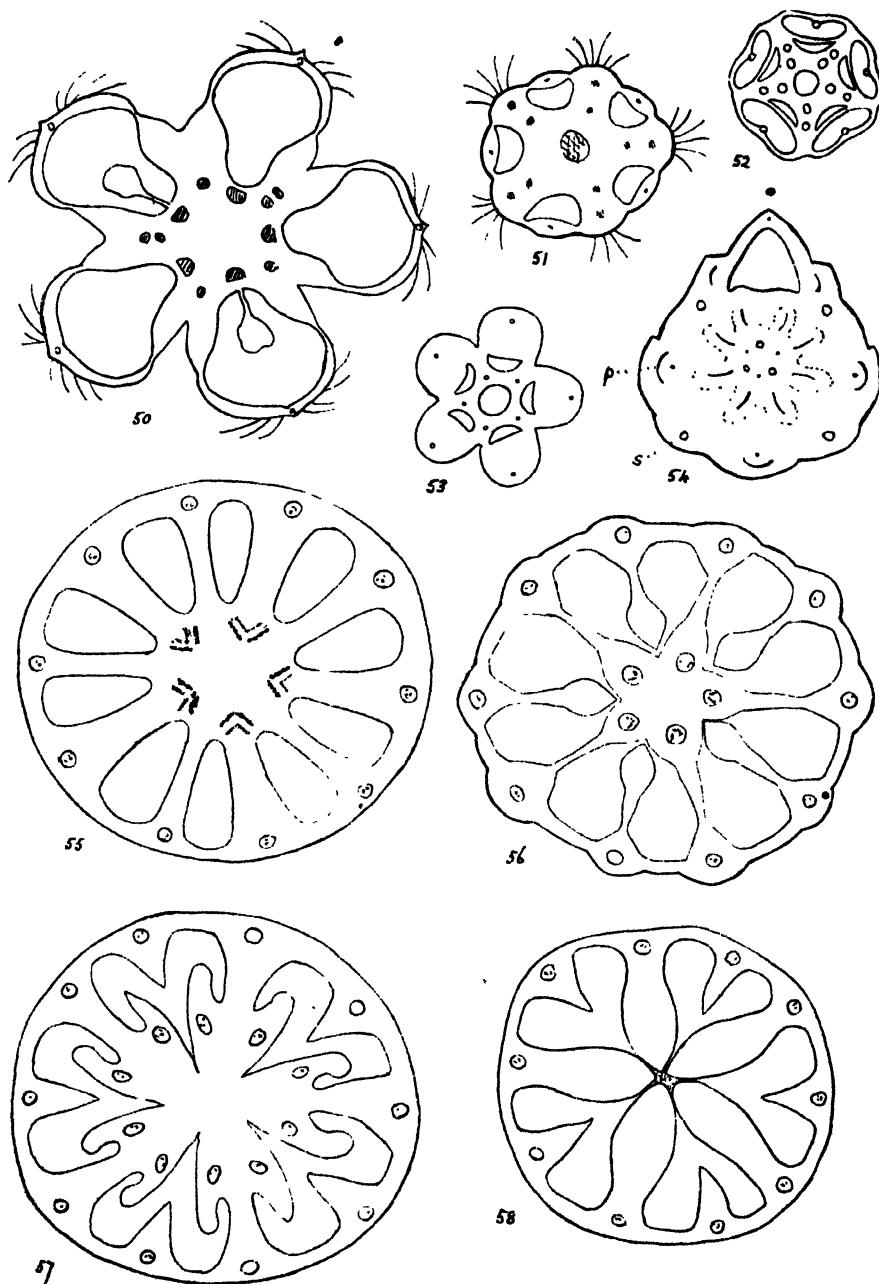
FIGS. 50–8.   50, 51. *Pelargonium* sp.   50. Transverse section of the ovary, showing the five carpels of the outer whorl which have retained their central position, and alternating with them those of the inner whorl which have bulged outwards to form the loculi.   51. Transverse section through the ' beak '

almost entire from the column formed of the solid carpels and central parenchyma. But in *Pelargonium* and *Geranium* the extreme thinness of the loculus wall at a point about midway between midrib and placentae leads to rupture along this line when the fruit dries. As a result only the front portion of each valve comes away, while the sides and placenta, together with the solid carpels, remain conjoined as a solid core. This separation at maturity of the body of a carpel from its placenta is decidedly rare. Though hitherto universally regarded as the explanation of what is observed to take place almost throughout the Cruciferae and in certain Papaveraceae, Fumariaceae, and Capparidaceae, we now know that this interpretation can no longer be accepted. Nor, as will presently appear, does such a conception apply in all types having the free central mode of placentation, though in the Caryophyllaceae we apparently have another genuine case (see above, p. 142).

Oxalidaceae. G 5 + 5. (Hitherto described as G 5 disposed round a prolongation of the axis, as in the Geraniaceae.)

Oxalis corniculata, L. G 5 + 5, similar in character and arrangement to the whorls of the Geraniaceae.

Tropaeolaceae. *Tropaeolum majus*, L., G 3 + 3. (Hitherto described as G 3.) (Fig. 54.)

The fact that occasionally flowers of species of *Tropaeolum* have been observed with a five-carpelled gynoecium, and that the carpels may then be situated (if we may take these cases to have been correctly recorded) sometimes opposite the sepals (Rohrbach), sometimes opposite the petals (Chatin), affords indication that the ancestral type may have had G 5 + 5, as has been shown to be the case in the allied families Geraniaceae and Oxalidaceae. According to Eichler,[1] the ovary base of *Tropaeolum majus* shows in cross-section six vascular cords, three strong, on the radius of the valves, alternating with three weak corresponding to the commissures. This alternate arrangement points to the survival of three members from each whorl. Now it is a well-known fact that some flowers of *Tropaeolum* are right-handed, some left-handed, and that whether the posterior valve is

of the same; the solid carpels have now become hairy, while the valve carpels have lost their hairs. 52. *Geranium sanguineum*, L. Transverse section through the 'beak'; the large areas between the valve carpel midribs and the loculi are occupied by stereome tissue. 53. *Erodium gruinum*, Soland. Transverse section of the 'beak'. 54. Transverse section through the flower of *Tropaeolum majus*, L., showing the six fibro-vascular bundles in the gynoecium, three strong alternating with three weak. 55–8. *Linum usitatissimum*, L. Transverse section of the ovary at different levels. 55. Section through the base, showing five broad septa formed by the fertile carpels alternating with the five narrower septa of the sterile carpels. 56. Section taken at a slightly higher level : the sterile carpels are being withdrawn from the centre. 57. Section through the middle region of the ovary : the sterile carpels are still farther withdrawn towards the ovary wall; the fertile carpels, each of which shows two placental bundles supplying a pair of ovules, still reach the central parenchyma. 58. Section through the top of the ovary above the ovuliferous region; the fertile carpels are now drawing apart at the centre. (Fig. 52 after Zimmermann, 53 after Hildebrand, 54 after Eichler; the rest original.)

[1] Blüthendiagramme, ii, Fig. 122, p. 299.

somewhat to the right or to the left of the median plane of the ovary is correlated with the position of the outer (front) sepal, which similarly lies sometimes to the right, sometimes to the left, of the median line. The case of the calyx involves no special problem, since the difference between the two forms is one merely of sequence and not of position of the sepals. But in the gynoecium this is not so, the valves in the right-handed flower corresponding in position to the partitions in a left-handed specimen, and vice versa. This change in orientation affords strong support to the view that the six vascular cords referred to above represent as many carpels, of which three (those belonging to one whorl) are more strongly developed than the alternate three (belonging to the other whorl). The presence of the two sets of carpels furnishes a ground plan from which it is possible to derive the one configuration or the other, according as the points upon which the forces producing carpel development act fall on the one set of radii or on the other.

Linaceae. *Linum usitatissimum*, L. G 5 fertile + 5 sterile, all solid or nearly so,[1] proper alternation with the staminal whorls. (Hitherto described as G 5, standing opposite the inner whorl of stamens.) (Figs. 55-8.)

A transverse section taken through the base of the ovary shows ten vascular bundles equally distributed round the ring of the ovary wall, and in line with them ten complete septa, of which five can be distinguished as rather broader than the other five with which they alternate (Fig. 55). At a slightly higher level the five narrower septa are seen becoming detached at the centre and assuming at their free ends a shape giving promise of the formation of placentae, which is not, however, realized,[2] while the alternate partitions, in which are seen the placental cords, are still united by some central tissue (Fig. 56). At a level somewhat higher still the incomplete septa (really the sterile inner carpel whorl) are withdrawn farther towards the ovary wall, the alternate and still complete septa (= the fertile outer whorl) have now developed typical arrow-head-shaped placentae, and each placental cord has divided into two to supply the ovules on either side (Fig. 57). At the apex of the ovary these latter five are also gradually withdrawn from the centre in the same manner as those of the inner whorl (Fig. 58), their vascular strands taper off, while the midribs of the inner carpels are prolonged upwards into the five styles, as was the case in Geraniaceae. It is to be noted that in *Linum* the obdiplostemonous condition is not marked, for the reason, no doubt, that here both carpel whorls are similar, and there is therefore no unequal outward thrust

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[1] As no sharp distinction can be drawn, as a rule, between a much contracted semi-solid and a slightly expanded solid carpel, classification of a border-line case such as *Linum* becomes largely a matter of convenience.

[2] Although a vascular strand is actually given off from the midrib and can be seen running horizontally in these narrow septa in the lower region of the ovary, it does not become functional.

on alternate stamens, such as occurs where one of the carpel whorls is composed of solid carpels which do not diverge outwards, and the other of valve carpels which bulge considerably, so that although actually the inner of the two they come to project farther out than the other whorl. The fruit dehisces by splitting of the ten vascular cords and septa, a fact difficult to explain on the old view that G = five valve carpels, but now readily intelligible seeing that the carpels have been shown to be ten in number and to be of the solid type, and therefore, as we have learnt from our examination of certain Leguminosae, capable of undergoing such a median split.

Begoniaceae. *Begonia* (ordinary type). G 3 semi-solid. (Hitherto the three-winged trilocular ovary, with three pairs of stigmas, has been regarded as composed of three valve carpels.) (Fig. 59.)

It can be seen by inspection of an ovary rendered semi-transparent in alcohol that a strong vascular cord (midrib) runs up the middle of each flat side of the ovary and gives off a system of lateral veins to one face of the wing on either hand. From this cord are also derived the placental bundles which lie on the same radius. There is no single continuous vein along the wing edge, although this is the position in which we should expect to find the midrib bundle. were the carpels actually of the valve type as has been supposed. As already stated, the wing venation system originates from the mid-line of the flat sides and extends thence outwards towards the wing edge, and not in the reverse direction. This scheme of venation, together with the central position of the ovules, affords clear proof of the *semi-solid* character of the carpels. At the summit of the ovary the midrib cords divide into two; these branches and those of the paired placental bundles diverge and run right and left towards the wing angles, with the result that the shank of each pair of stigmas standing over the fruit angles is supplied, not by the *whole* vascular system of *one* carpel, but by *half* the system of each of *two* adjacent carpels. This mode of formation is not uncommon in other families. and is well seen, e. g., in *Drosera* (see Fig. 38). We meet here, in fact, with the very phenomenon which has been so often invoked by systematists in the case of *valve* carpels, admittedly, however, not on any actual evidence, but as a theoretical necessity in order to account for the position of the supposed commissural stigma. Such splitting of the stigma is not compatible with the valve carpel configuration, and, as we now know, the necessity for postulating its occurrence in these cases no longer exists. This behaviour is on the other hand entirely in keeping with the character of the solid and semi-solid carpels, whose midrib is not infrequently composed of a double bundle.

Lythraceae. G n + n. (Hitherto described as G n, n varying from 1 to 6.) (Figs. 60–3.)

Cuphea ignea, DC. G 2 (valve) + 2 (solid). This genus, again, provides most excellent material for illustrating the present standpoint. The
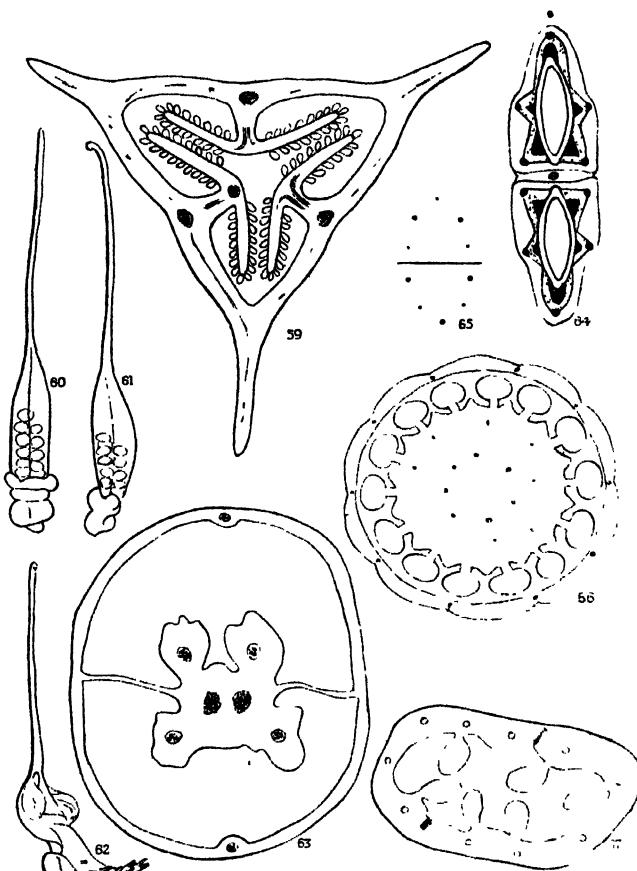
FIGS. 59-67. 59. *Begonia* sp. Transverse section of the ovary, showing the three semi-solid carpels.
60-3. *Cuphea ignea*, DC. 60. The ovary,' which has been rendered partially transparent, shows
the central column (composed of two solid carpels) on which the ovules are borne. 61. The same
seen from the side : the central vertical line indicates one of the septa, which is seen through the semi-
transparent wall. 62. The ripe fruit after the ovary wall has been torn asunder by the elongating
column of the solid fertile carpels. 63. Transverse section of the ovary ; in the centre the two
separate vascular cords (midribs) of the two solid carpels with massive placentae, alternating with the
two valve carpels which form the septa. 64. Transverse section of the ovary of *Hydrocotyle vulgaris*,
L., showing the ten carpels (see text). 65. Scheme showing how it comes about that in genera in
which the commissural and carinal ribs (= the two whorls of carpels) develop unequally the one meri-
carp will not be the identical mirror-image of the other. 66. *Primula vulgaris*, Huds. (Polyanthus).
Transverse section of the ovary, showing the ten sterile valve carpels forming the wall and seven
solid fertile carpels composing the central column. 67. *Gentiana Cruciata*, L. Transverse section of
the ovary, showing that each of the two fruit valves is composed of five carpels, the centre one being
sterile, and any or all of the others fertile. (Fig. 64 after Drude ; the rest original.)

membranous wall of the ovary is almost transparent, and if placed in alcohol shows clearly a single unbranched vascular cord running up both *median* faces from base to apex, the two strands being continued up into the single style (Fig. 60). In a transverse section these two bundles (mid-ribs) are seen cut across, and midway between them a narrow septum stretches on each side from the ovary wall to a central column of tissue in which are seen two strong vascular cords in the *lateral* plane of the flower and four bulky placentae which are supplied from these latter cords (Fig. 63). This central column originally extends only part of the way up the ovary cavity, but after fertilization it elongates greatly, bursting through the delicate ovary wall, which is torn to shreds (Fig. 62). The idea (inevitable on the orthodox view of G 2) of carpels whose united edges thus gore their own enclosing bodies strikes one as almost rivalling the fable of the man who could jump down his own throat! On the poly-morphic view there is no difficulty. An outer whorl of two sterile valve carpels constitutes the ovary wall and is prolonged upwards to form the style; alternating with these are two solid fertile carpels which have kept their central position.

Umbelliferae. G 5 + 5. (Hitherto described as G 2.) (Figs. 64 and 65.)

The ovary in most Umbelliferae is marked by ten ribs or ridges, five being present in each mericarp. On the radius of each rib is a fibro-vascular cord, the whole ten running a separate and parallel course up the ovary. The two loculi are described as having each originally two ovules, which, according to von Roper[1] and Sieler,[2] are attached one to each edge of each carpel (so called). These ovules must, therefore, be supplied by the four cords nearest to and on each side of the lateral plane (= plane of separation of the mericarps) (Fig. 64). The carpels which these cords represent alone are fertile; the others, though similar in form, have become sterile. Although the central column (carpophore) is in most cases composed merely of mechanical and ground tissue, the fact that in a few genera some tracheides are traceable suggests that in the ancestral type a more considerable prolongation of the axis occupied the centre of the carpel whorl, and that a diminution in bulk, especially in the lateral plane, at a later phylogenetic stage resulted in the formation of an apparent com-missure and the final split into the two mericarps. Although the ten carpels have the appearance of standing in a single whorl they actually form two whorls of five, and herein lies the explanation of the hetero-morphism of the twin mericarps in some genera. For where one whorl is more strongly developed than the other it follows that one mericarp will have three strong ribs opposite the sepals (carinal), and two weak (commissural), whilst in the other these numbers will be reversed, a con-struction difficult to account for on the supposition of G 2, but quite

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[1] Bot. Zeit., 1856, p. 484.　　　Ibid., 1870, p. 381.

compatible with a ground plan of G 5 + 5.   Each mericarp, therefore, represents a consolidated group of fertile and sterile carpels, a formation which we shall see exemplified again in the case of the fruit valves in *Gentiana.*

Primulaceae.   *Primula, Cyclamen.*   G 10 + 10 or fewer, carpels always dimorphic.   (Hitherto regarded as G 5.)   (Fig. 66.)

Free-central placentation here arises in a manner somewhat different from that described above in the Caryophyllaceae.   There appears first an outer ring of sterile carpels, usually ten in number, but sometimes more or fewer, and often of unequal width, which, bending outwards, form the wall of the ovary, their vascular cords (midribs) being prolonged upwards into the single hollow style.   Within the ovary cavity a central column of tissue *with a well-defined surface layer* is prolonged for a short distance above the level of origin of the valve carpels, and usually shows a reduced number (seven or eight) of vascular cords corresponding to as many fertile carpels which have failed to achieve the outward curve, and retain their central position.   At a slightly higher level there is a considerable increase of ground tissue, and at the same time these cords break up, each supplying a pair of placentae, and each placenta bearing a row of ovules, unless perchance one here or there gets crowded out. *There is here no tearing of the tissue of the members of an inner carpel whorl* resulting in the separation of the midribs from the placentae, as is observed in the Caryophyllaceae, the placentiferous column in the Primulaceae being covered throughout by a distinct limiting layer.   In the light of the facts described for the various other families already considered, there is little doubt that this placentiferous column is formed *not merely of placentae, but of whole conjoined carpels,* which are of the solid type and fertile.   On the current view that the carpel valves forming the ovary wall (which even so must be held to be ten and not five) have become separated from their placental edges, which have all united to form the central column, it is difficult to account for the case recorded by Duchartre,[1] in which, although the ovary wall was absent, the central placentiferous column was developed in a flower of *Dodecatheon.*   To hold that the edges of a carpel are able to be present without the body of the carpel of which they are supposed to form the confines is to strain morphological conceptions somewhat severely.   But that an outer whorl of whole carpels (for as such the ovary wall is to be regarded on the polymorphic view) should in an isolated instance have undergone suppression, merely offers one more example of a phenomenon which is not of uncommon occurrence elsewhere.

Gentianaceae.   *Gentiana.*   G 6–10 or more.   (Hitherto described as G 2.)   (Fig. 67.)

The ovary in *Gentiana* is unilocular, with several (? 4–8) parietal

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[1] Ann. sci. nat., Bot., 3ᵉ sér., ii, p. 289, 1844.

placental cords, each supplying 1–2 (? or more) rows of ovules. It bears a single style with bilobed stigma, the lobes standing over the two lateral valves, into which the fruit splits when ripe. The number of these vascular cords varies with the species: six were found in *G. acaulis*, L., ten in *G. Cruciata*, L., and more than ten in *G. decumbens*, L. In *G. acaulis* one of these vascular strands is situated in the middle of each fruit valve, and one towards the back and front margins respectively. These latter four supply the funicles of a corresponding number of bands of ovules. A transverse section of *G. Cruciata* showed ten vascular cords distributed round the ring of the ovary wall, five occurring in each of the two portions corresponding to a fruit valve (Fig. 67). The limits of the fruit valves are indicated by two indentations flanked by sclerenchyma on opposite sides of the ovary cavity. In any one section some six or seven of these bundles may be seen to have ovules springing from[1] them, want of space alone presumably preventing the development of ovules from all but the mid-lateral cords. Evidently the six or ten cords, as the case may be, correspond to whole carpels, all of which are solid, and all but two actually or potentially fertile. These two bundles alone, as a rule, continue up to the top, and supply the two stigma lobes. The fruit valves are thus shown to be not *single carpel* valves, but *compound*[2] valves composed of a group of five carpels in *G. Cruciata* and of three in *G. acaulis*, which become detached in one piece.

Gramineae. Ground plan G 3 + 3, not however ordinarily, and possibly never realized in full in living genera. (Hitherto variously regarded as G 1 or G 2 except in the Bamboo group, where G is held to = 3).

The Gramineae present special difficulties in the analysis of the gynoecium, owing to extreme reduction in development of the vascular system, which in many carpels approaches, if it has not actually reached, vanishing point.[3] Of the six carpels the outer three lie on the same radii as the outer stamens (in most cases the only ones present). They are apparently always sterile, but bear the styles (when present) and stigmas. Except in the Bamboo group, in a few genera, as e. g. *Hordeum* and in the unique case of *Nardus*, the unpaired front member of the trio, if it can be deemed to be present,[4] shows no development of vascular tissue. The two postero-lateral members either form two distinct sessile stigmas or they are prolonged upwards for a short (*Oryza*), medium (*Coix*), or very considerable

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[1] Either directly or from a lateral branch, for the placentation shows an approach to the superficial type in all the forms examined.

[2] Met with also, as already described, in the Umbelliferae and again in *Eschscholzia*, where, however, they consist of *two* types of carpels, valve and solid alternating with each other.

[3] For carpels giving rise to a fertile ovary such suppression of the vascular tissue is most exceptional if not unique, though it is characteristic of the rudimentary pistil present in the male flower of many species with unisexual flowers. *Zanthorhiza apiifolia*, L'Hérit., is an interesting case in point.

[4] In these circumstances decisive proof is not obtainable.

(*Zea*) distance as a single style, which sooner or later bifurcates. When a style is formed, the two carpels each show a single thread-like vascular bundle (midrib) which is continued up the length of the style and generally into the stigma branches.   When a style is absent the paired carpels may still each develop a vascular bundle running up into the stigmas (*Panicum*, *Setaria*, *Gynerium*), or these bundles may also be wanting.   The ovary wall is then destitute of vascular tissue unless the placental cord is developed for some distance up the back face.   The twin stigmas are situated in the same position as in the preceding case, but here vascular elements are of course lacking.

The inner carpel whorl is represented by a single member which is fertile but not stigmatic, and posterior in position.   In some genera it terminates above in a blunt projection seen between the twin stigmas of the two postero-lateral carpels of the outer whorl (*Oryza*, *Anthoxanthum*, *Setaria*).   The placental cord constitutes the midrib, and when strongly developed may reach to the top of the ovary (*Oryza*).   In *Avena*, *Elymus*, *Triticum*, it extends about half-way up, but in a large number of genera, of which *Poa annua*, L., may be taken as an example, in which the ovule is borne at the base of the ovary, the vascular tissue ceases at this level.   In *Hordeum*, according to Schuster,[1] a transverse section of the ovary shows four vascular strands owing to the fact that the unpaired front carpel of the outer whorl develops a weak fibro-vascular bundle, a rare occurrence outside the Bamboo group.   This fact in itself seems to me fatal to the proposition which Schuster seeks to establish, viz. that the formula for all Grasses is G 3. For if, as he holds, the placental cord represents the junction-line between two carpels, then in such a case as *Oryza*, where the placental cord extends right to the top of the ovary, it follows that we must suppose it possible that two adjacent carpels can form a bulky placental cord throughout their length where their edges meet on the one side, and merge imperceptibly into one another without forming any vascular tissue or even a demarcation line, on the other.   But, in fact, this interpretation breaks down on other points.   It passes belief, for example, that the bulky mass of vascular tissue forming the placenta in such a type as *Zea* can be derived from the few minute vessels of the midribs of the two stigma-bearing carpels on either side.   In the Bamboos the three carpels of the outer whorl are all equally developed, ending above in a single style with three stigmas or in three completely separate styles and stigmas (*Bambusa Tulda*, Roxb.).   In the unique case of *Nardus stricta*, L., it is the unpaired front carpel which furnishes the single vascular bundle to the style, and although it is highly probable that in this plant only this member of the outer whorl and the inner posterior fertile carpel are present, it has been shown above that in this family absence of vascular tissue does not always imply total absence of the carpel itself.   This veinless condition, coupled with the unique character

[1] Über die Morphologie der Grasblüte. Flora, 1910.

of the fruit, leads one to speculate whether the contraction, which has resulted in the extreme case in suppression of the vascular tissue, may not have been a factor in bringing about the fusion of testa and pericarp.

Eriocaulaceae (Figs. 68 and 69). In this family we have another excellent illustration of the lengths to which it is necessary to go in order to fit the facts into a scheme admitting of only a single carpel type. According to current views *Eriocaulon, Paepalanthus*, and their allies are held to have G 3 (or 2), with stigmas normally placed in *Eriocaulon* and commissural in *Paepalanthus*, which is moreover furnished with peculiar appendages not found in *Eriocaulon*.

Paepalanthus. G 3 + 3 (or 2 + 2). It is to be noted that the (so-called) commissural stigmas are frequently forked (*Paepalanthus vellozioides*, Koern., *Blastocaulon rupestre*, Ruhl., *Lachnocaulon anceps*, Morong., see Fig. 68), and that the (so-called) appendages occupy the position, and although of different form have the appearance, characteristic of genuine stigmas. In order to explain this construction on the supposition of G 3, Eichler [1] finds it necessary to assume (1) that each carpel member became split at its apex into three segments; (2) that adjacent lateral segments of neighbouring carpels became more or less fused (after the manner conceived by systematists as occurring in many of the Rhoeadales, as e. g. *Papaver*), and thus produced the functional and often forked commissural structures; (3) that the middle segment became transformed into a functionless appendage of quite different appearance. There is, of course, no evidence whatever of any such processes having taken place. Moreover, when the commissural stigmas are wanting, the appendages are formed into proper stigmas, and in the rudimentary ovary of the male flower they are the only ones present. Notwithstanding the conclusive nature of these facts, Ruhland,[2] who takes exception to Eichler's view on the ground that it is too complicated, considers it best to regard what is taken to be the inner whorl of structures (appendages) as outgrowths comparable with the corona on a corolla. He holds Koernicke to be correct in dissenting from the suggestion put forward by von Martius that these outgrowths indicate the presence of an inner carpel whorl,[3] since the two whorls would then be superposed. It is, however, quite evident that two whorls are present, an outer one whose stigmas have ceased to function and an inner whorl composed (presumably)[4] of semi-solid carpels, hence the two-pronged form of

[1] Blüthendiagramme, ii, p. 138.

[2] Pflanzenreich, iv, 30, p. 17, 1903.

[3] Also so interpreted by Le Maout and Decaisne, who, however, do not deal with the new difficulty, viz. superposition, which their suggestion, as it stands, creates.

[4] Owing to lack of suitable material, this point, though not in doubt, could not actually be verified. For the same reason it cannot be stated with absolute certainty that the outer whorl does not consist of true valve carpels but of solid carpels reduced to their vascular cords. At the same time the general applicability of the conclusion arrived at in the case of the Liliaceae (see later, p. 164), and the fact that dehiscence is loculicidal, leave little doubt that this will prove to be the case.

their stigmas in some species.   In the rudimentary gynoecium of the male
flower it might well be expected that the inner whorl would not come to
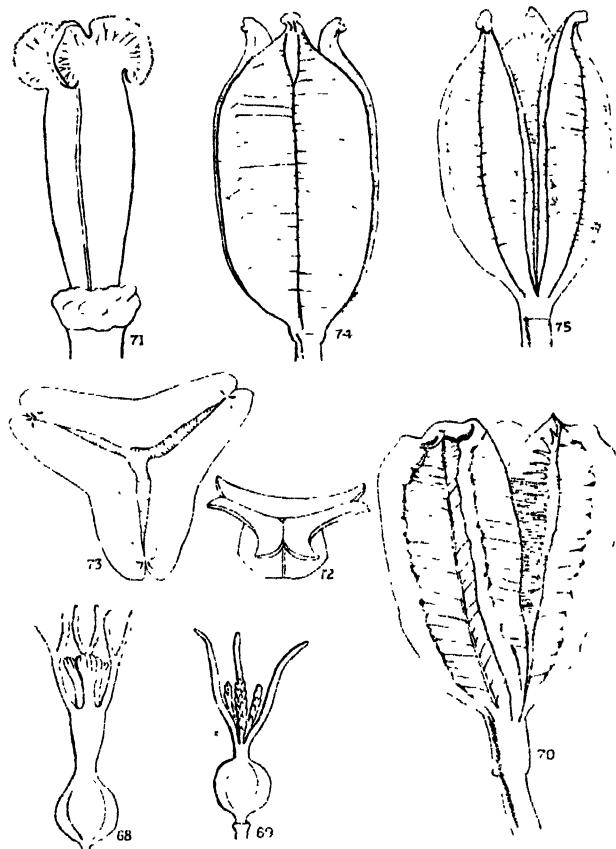
FIGS. 68–75.   68. *Lachnocaulon anceps*, Morong.   Ovary with dimorphic styles and stigmas, the
commissural stigmas forked.   69. *Paepalanthus brachypus*, Kunth.   The same, with simple commissural
stigmas.   70. *Fritillaria Imperialis*, L.   Ripe fruit after dehiscence, showing the venation system
arising from the midribs of the semi-solid carpels, the splitting of the midrib at dehiscence, and the
tearing in halves of the finger-like processes projecting into the loculus.   71–5. *Tulipa Gesneriana*, L.
71. Young fruit injected to show the venation of the stigmas.   72. Transverse section through the
stigma region.   73. The same more highly magnified, showing the large number of scattered vascular
bundles (indicated by dots) derived from the semi-solid carpels and the one or two (indicated by short
lines) at each end of each stigma derived from the solid carpels.   74. Ripe fruit after dehiscence,
showing in the centre the venation system arising from the midrib of one of the semi-solid carpels
(fruit valve) and right and left the smaller system arising from the solid carpel.   The semi-solid
carpel midrib forks towards the top of the valve.   75. The same turned so as to bring one of the fruit
angles into front view, showing splitting of the solid carpel.   (Figs. 68, 69, after Ruhland ; the rest
original.)

development, and consequently that the outer whorl should then produce the
functional type of stigma.

Liliaceae (Figs. 70–83).   In most members of this family the ovary
when cut across shows three strong vascular cords situated opposite the

loculi at the angles of the usually trigonous ovary. From the three flattened sides arise the septa which meet in the centre, in each of which is to be seen a narrow median slit—the lumen of a septal gland (extra-floral nectary). These glands are not present in the genera *Fritillaria*, *Lilium*, and *Tulipa*, whose solid septa show at their outer ends an additional strong vascular cord comparable with those opposite the loculi. These three genera form a most instructive series and afford a clue to the general Liliaceous ground plan.

Fritillaria, Lilium, Tulipa. G 3 + 3. (Hitherto described, like all trimerous Liliaceae, as having only G 3.)

Fritillaria Imperialis, L. (Crown Imperial). (Figs. 70 and 76.) The six-sided, six-winged fruit shows in cross-section a ring of six vascular cords, one being situated in the middle of each flat side. These six cords give rise to six systems of venation and constitute the midribs of six semi-solid carpels. Of these carpels three, which are smaller and without placentae, are opposite the loculi, and their midrib bundles are continued up into the three styles, which are connate. Alternating with these sterile carpels are three somewhat larger, fertile, and of the T-shape characteristic of the semi-solid type when the tissue over the midrib is not withdrawn from the centre but forms a complete septum (corresponding to the long arm of the T). Only, however, for the very short distance extending from the extreme base of the ovary to a level just above that at which the vascular cords of the sterile carpels run outwards and the loculi make their appearance do the septa form at the centre a continuous tissue. Throughout the rest of the length of the ovary their central ends, although in contact, are delimited, the surfaces being indicated by the double layer of epidermal cells seen in a transverse section extending from the mid-point of the section to the three loculi as a three-rayed boundary line (Fig. 76). Each of the six vascular cords (midribs) gives rise, right and left, to numerous parallel lateral veins which extend to the line of junction of each carpel with its neighbour (Fig. 70). Here these laterals give rise to a system of arches from which a few free endings are prolonged a short distance into the wings formed as a post-fertilization development by the growth outwards of each pair of fused carpel margins. Each wing thus receives some elements from two separate vascular systems derived from the carpels on either side. This disposition of the vascular tissue is wholly irreconcilable with the old view that the region represented (as here interpreted) by one whole semi-solid sterile carpel, together with half of each semi-solid fertile carpel on either side, constitutes a single valve carpel. It is on the other hand entirely in accord with a six-carpelled scheme of construction of the type described above. On their inner face the sterile carpels show a slight bulging into the loculus of the tissue over the midrib. At the flowering stage these arcs are seen to have an epidermis of different

character from that lining the rest of the loculus. The cells are large, almost papilliform, with a thick, deeply staining outer wall; whereas, elsewhere around the loculus, the epidermal cells become flattened tangentially, present an even surface, and stain like the rest of the tissue. So sharply indeed is the point at which the change occurs defined, that it seems clear that we are here able to identify the exact spot at which the epidermis of the sterile carpel ends and that of its fertile neighbour follows on. This view that the extent of this deeply stained arc represents the width of the sterile carpel on its inner face receives support from the following fact. As the fruit matures there develops from each of these regions a vertical row of finger-like processes projecting into the ovary cavity between the two columns of seeds occupying each loculus. These late-formed outgrowths may be compared to the region of the replum internal to the vascular cord in a Crucifer, for both result from a resumption of growth by a carpel that has undergone consolidation, and it may be supposed that, in the one case as in the other, the whole breadth of the carpel is involved. If this inference is correct, it follows that the point of junction of two adjacent carpels on their inner face does not lie on the same radial line as the corresponding point on their outer surface, for the outer epidermis of each carpel presumably extends from wing edge to wing edge, whereas only a part of the region of inner epidermis enclosed by the radii lying in the plane of the wings is made up of the deeply staining cells described above. This disparity becomes much more pronounced after fertilization, as the ovary becomes enlarged to many times its original size. It also persists, apparently, up to and beyond the base of the style, for although it is the sterile carpels which are prolonged upwards to furnish the outer epidermis and vascular strands, the epidermis lining the stylar canal at this level shows an arc of papilliform cells centred over the disappearing midribs of the fertile carpels. From these points the papillae spread out on either side until at a higher level they meet laterally and form a complete lining to the canal. The mode of dehiscence of the fruit is loculicidal, and is brought about by the splitting asunder of the double bundle forming the midribs of the sterile carpels in the line of the finger-like outgrowths, which thus become torn lengthwise in two (see Fig. 70). In *F. Meleagris*, L., the ovary when cut across shows the bluntly triangular outline usual in the Liliaceae, with the loculi opposite the angles and the septa opposite the flat sides. As in *F. Imperialis*, there are six vascular cords, and the three fertile carpels are, as in that species, semi-solid; but the three sterile members have undergone a further degree of consolidation and are of the typical solid type. On their inner face they show the same convex arc of deeply staining epidermal cells delimiting them from their fertile neighbours as was noticed in the larger species (Fig. 77), but on the outer surface of the ovary their boundaries are not defined. It is clear, however, from the distribution of

the vascular tissue that they have no considerable lateral extension such as is seen in *F. Imperialis*. At the same time, the fact that there is no external longitudinal furrow at the angles of the gynoecium, such as occurs in many Liliaceae, suggests that at least some portion of the angle tissue is derived from the solid members, and that they furnish part of the outer epidermis of the ovary as well as of the inner epidermis of the loculus.

Tulipa Gesneriana, L. (Garden Tulip). (Figs. 71–5 and 78 and 79.) In respect of the general contour of the cross-section, the number and disposition of the vascular cords, the relative positions of the loculi and septa, the ovary of the Garden Tulip is very similar to that of *Fritillaria Meleagris*. It differs from the latter plant, however, in one or two important particulars. Thus the delimitation of the central surfaces of the fertile carpels (= septa) is not accomplished so soon as in *Fritillaria*, a cross-section of the ovary showing continuous tissue in the centre for a considerable distance above the base. The even outline of the section is broken by a notch at each angle due to a longitudinal furrow which is absent in *Fritillaria*. On the other hand, the convex arc, with its deep-stained epidermal cells, projecting from the inner face of the ovary wall into the loculus opposite the angle, which is characteristic in *Fritillaria*, is not found in the Tulip. Instead, it is to be observed that the ordinary epidermal cells lining the two sides of the loculus are discontinuous in the centre line, and turn outwards at a right angle so as to traverse the thickness of the ovary wall almost up to the vascular cord (midrib of the solid carpel) (Fig. 78). At the point where the right angled turn is made ·a few epidermal cells on either side of the line of cleavage grow out into the loculus, forming long hairs which fit between the two columns of seeds in the manner of the more solid single structure which occurs in *Fritillaria* (Fig. 75). These hairs furnish us with a clue to what has taken place. They are found at this point because just here we have in the first place the meeting of two actual edges, and secondly· the presence of a cavity (the loculus) which permits of hair formation. The sterile carpels in the Tulip have obviously suffered a further degree of reduction. They have retained their vascular cord (midrib), but they have been engulfed fore and aft. by the semi-solid carpels. They neither reach to the outer surface of the ovary wall nor to the boundary of the loculus. The notch which appears in the outline of the section at the angles indicates the point of junction of the semi-solid carpels as they met and closed over the intervening solid member. The same enveloping process occurred on the inner surface, but here the traces of the method of obliteration are more obvious. Though the edges of the semi-solid carpels have met, they have not coalesced. The contact surfaces are still visible, and the presence of the hairs is corroborative evidence of their true nature. But the solid carpel has not wholly disappeared, though it is now completely buried and has lost its superficial tissue layers. Enough still remains to enable us to trace its

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history.   The sessile stigmas form three bilobed crests surmounting the three flat sides of the ovary and protruding over the edges at the angles (Fig. 71).   In a transverse section taken through this region they appear as three semilunar structures *centred in line with the septa* (flat sides) (Fig. 72), without a distinct midrib but with numerous scattered fibro-vascular bundles (Fig. 73).   These bundles are derived from the splitting up of the double bundle (midrib) of the semi-solid carpels, the protruding down-curving stigma edges alone receiving one or two small branches (indicated in Fig. 73 by short lines where the lobes touch) from the midribs of the three solid carpels (at the angles).   This can be very prettily shown by injecting the ovary by placing the flower stalk in an aqueous solution of eosin.   The fluid is drawn much more rapidly up the almost unbranched vascular cords of the solid carpels than up the divided bundles (midribs) of the semi-solid carpels with their numerous ultimate ramifications.   By this method a preparation can be obtained in which the few (generally one or two) bundles at the outer borders of the stigma are stained, while the rest, i. e. those derived from the semi-solid carpels, are uncoloured.   The position and structure of the stigmas is at once comprehensible when it is recognized that they are formed almost entirely from the semi-solid carpels.   On the old view that $G = 3$, though not generally so described they could only be regarded as commissural and hence would present the usual difficulty inherent in all such cases.   The true significance of another puzzling though seemingly trivial characteristic of the fruit also now becomes apparent.   When ripe the capsule very usually shows, about half-way or two-thirds up its length, a distinct bifurcation of the main vascular cord in the middle of the flat sides (Fig. 74).   On current views this appearance would be ascribed to separation of the conjoined placental edges of the valve carpels on either side. This explanation does not, however, account for the broad strip of non-vascular tissue intervening between the two arms of the fork.   Now that we know each flat side to be composed of one semi-solid carpel, the separation and divergence of the twin bundles which compose its midrib is readily comprehensible, and is comparable to the similar bifurcation which takes place, although not visible externally, at a much higher level—just below the stigmas in the solid carpels.

Tulipa sylvestris, L. (Fig. 79), differs chiefly from *T. Gesneriana* in that the semi-solid carpels are not continuous at the centre, even at the base of the ovary, and that the stigmas are much less developed.   The fact that in this species the vascular bundles of the semi-solid carpels cease at a slightly lower level than those of the solid carpels makes it possible here also, by cutting a transverse section through the stigma apex, easily to observe the extent of the area supplied respectively by the vascular bundles of the two carpel types.

Lilium Martagon, L. (Fig. 80).   The general plan of construction is

similar to that of the Tulip, but here delimitation of the centre surfaces of the semi-solid carpels is not apparent, the placentae forming a continuous tissue throughout the greater part, if not the whole length of the ovary.
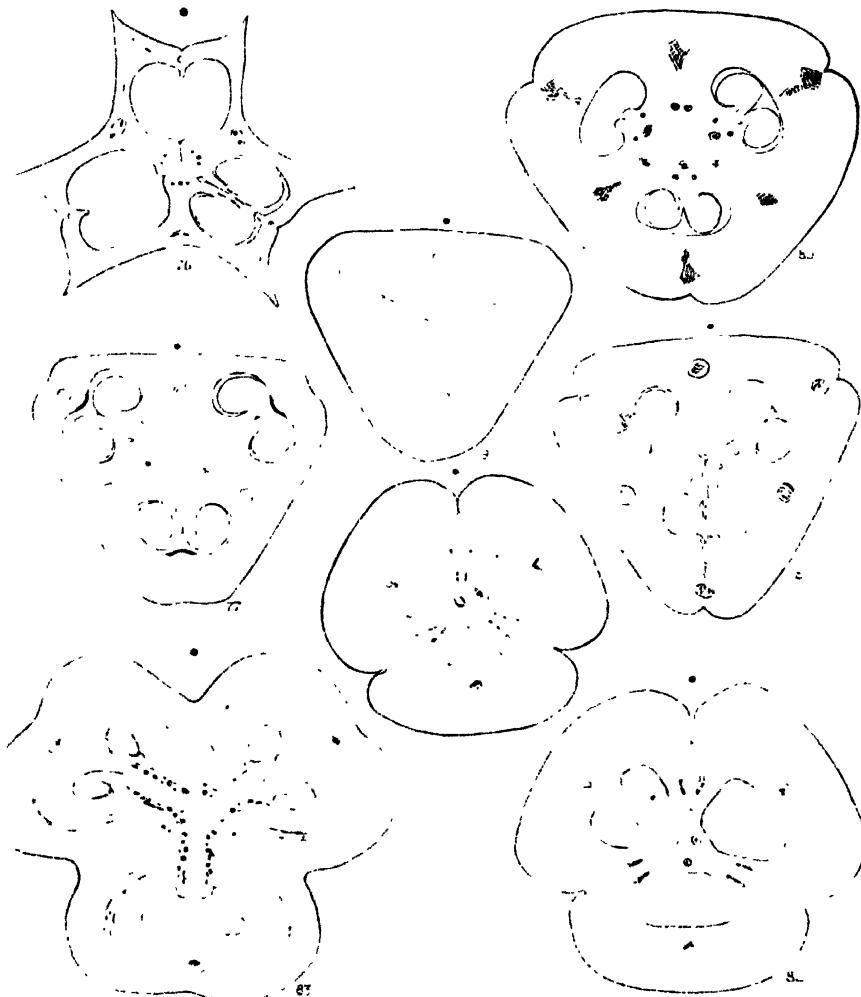
FIGS. 76–83. The ovaries of various members of the Liliaceae seen in transverse section (for explanation see text). 76. *Fritillaria Imperialis*, L. 77. *F. Meleagris*, L. 78. *Tulipa Gesneriana*, L. 79. *Tulipa sylvestris*, L. Section through base, loculi just appearing. 80. *Lilium Martagon*, L. 81, 82. *Polygonatum multiflorum*, All. 81. Section through the base. 82. Section through the ovuliferous region. 83. *Yucca flaccida*, Haw.

Further, all trace is now lost of the process by which the solid carpels have become hidden from sight on the side bordering the loculus. Their very large vascular cords are covered by a few layers of ground tissue and an epidermal layer in which no indication is visible either of a line of cleavage

or of the formation of hairs.   The semi-solid carpels have in fact not merely closed over and as it were buried the last remnant (midrib) of the reduced carpels, but have so completely coalesced that no sign of the fusion remains on this face.

The successive stages in the processes of consolidation and obliteration observable in the three genera under consideration will be seen at a glance from the following summarized statement :

Fritillaria Imperialis, L.   G = 3 small sterile semi-solid carpels + 3 somewhat larger semi-solid fertile carpels.

F. Meleagris, L.   G = 3 sterile solid carpels with inner and outer epidermis as well as vascular cord + 3 semi-solid fertile carpels.

Tulipa Gesneriana, L.; *T. sylvestris*, L.   G = 3 sterile solid carpels reduced to their vascular cords + 3 semi-solid fertile carpels.   The junction of the semi-solid carpels which have engulfed the others is visible on both inner and outer surfaces.

Lilium Martagon, L.   As *Tulipa*, but coalescence of semi-solid carpels on their inner surfaces is complete, leaving no trace of fusion.

In all three genera it is the *outer* whorl which has undergone consolidation and reduction of bulk.   This is proved not only by its position opposite the sepals, but also by the fact that in sections taken immediately below the loculi the midribs of the sterile members can be seen to lie farther from the centre than the vascular cords of the fertile members.

The presence of two whorls of carpels having thus been established for the few genera in which septal glands are absent, it is now necessary to consider the very large section of the family possessing these nectar glands and at the same time lacking the strong vascular cords (midribs) on the corresponding radii, which occur in the glandless genera.   As examples we may take a few types selected at random.   A transverse section through the base of the ovary of *Polygonatum* shows the usual outcurving of three vascular cords in line with the loculi, the other bundles remaining grouped at the centre.   At a slightly higher level another three bundles from the latter group turn out horizontally along the septa.   These bundles are seen to be double (Fig. 81), and each shortly separates into two strands which turn upwards abreast of each other.   Between these twin strands, which may subdivide further, the lumen of the gland makes its appearance in the midline of the septum, at the central end of which only the placental bundles now remain (Fig. 82).   It is evident that we have here the same ground plan as in such a type as *Lilium*, but the development of the gland has been accompanied by the break-up of the vascular cord (midrib) of each of the three inner carpels, thus providing equally for the supply to the secretory tissue on both sides of the lumen ; with the result, however, that except at the extreme base of the ovary the presence of the inner whorl escapes recognition, it not being obvious from inspection of sections taken at the gland

level that the double set of bundles in the septum arises from a single vascular cord—the midrib. No line of junction can be made out between adjacent carpels. Consequently, though it may be safely asserted that the outer whorl is sterile and the inner whorl fertile and semi-solid, as in the glandless types, the precise form of the outer whorl cannot be stated with the same certainty. But the trigonous form of the ovary suggests that these carpels have no lateral extension on either side of the midrib, i. e. they are solid.

The ovary of *Yucca flaccida*, Haw. (Fig. 83), presents a much more substantial structure, but the scheme of construction is at once seen to be on the same lines as before. A cross-section through the base of the ovary shows the outer whorl of solid carpels not yet completely withdrawn from the centre, but still forming projections into the loculus, an appearance frequently observable in the case of solid carpels in this[1] and other families,[2] which, on the old view that all carpels are of the valve type, was explained as due to false partitions arising from the midribs.[3] The break-up of the midribs of the semi-solid carpels is more pronounced than in *Polygonatum*, and the resulting collection of bundles remains spread out round the central arrow-head-shaped ends of the semi-solid carpels, instead of extending outward along the septa. In very many genera, indeed, it appears to be the rule for these vascular strands not to embrace the septal glands in the manner seen in *Polygonatum*, but to remain in this central position. In such cases, of which we may take *Scilla nutans*, Sm., as an example, they are only seen as three solid cords alternating with the outer three *at a level just beneath the loculus*. Above this region they break up into the paired placental bundles, and their real nature ceases to be apparent. But the fact that the fertile carpels are semi-solid is, on the other hand, more clearly seen in *Yucca* than in *Polygonatum*, owing to the abundance of the lateral veins to which the vascular strands give rise. These veins can be followed in many sections, sweeping round the curve of the loculus towards the midrib of the solid carpels, which here, as in all types so far examined except *Fritillaria Imperialis*, is itself either unbranched or forms only a very rudimentary branch system. A beautiful instance of the contrast between the unbranched vascular cords of the outer solid carpels and the well-developed reticulations which may arise from the inner semi-solid whorl, already noted in the glandless genera, is to be found also in the genus *Veltheimia*, in which the thin parchment-like wall of the ripe 3-winged fruit becomes semi-transparent, so that the complete network can easily be viewed in its entirety. In the solid nature of the outer carpel whorl we find an explanation of the slightly emarginate or bilobed form of stigma occurring in many *Yucca* species—a character not met with, so far as appears, in a valve carpel.

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[1] See e. g. *Dichopogon strictus*, Baker (Pflanzenfamilien, ii, 5, p. 34, Fig. 22, D).
[2] As e. g. in Linaceae.
[3] A conception incorrectly implying protrusion instead of retraction.

There is, indeed, much to suggest that within this single genus might be found a series as interesting from the present point of view as any among the Liliaceae, for the fruit exhibits precisely the kind of variations (capsulate and baccate, dehiscent and indehiscent, when dehiscent loculicidal and septicidal, six-celled and three-celled, with core and without core) which are likely to be associated with varying degrees of consolidation and reduction. But material for such an investigation is not easily come by, except in its native country.

In all the genera hitherto dealt with the trend has been in the direction of more or less consolidation of all the carpels with retention of both whorls. In certain genera of the section Melanthioideae we appear to meet with the converse condition—no consolidation of the outer whorl, which retains the valve form, but reduction of the total number by complete disappearance of the inner whorl, as e. g. in *Melanthium virginicum*, L., where $G = 3$ typical fertile stigma-bearing valve carpels.

The considerations set forth above not only throw light upon the number of carpels present, but they lead also to the following further conclusions concerning most members of the Liliaceae :

1. That the septa are not the congenitally fused margins of two adjacent carpels, but are composed of the middle region of individual semi-solid carpels.

2. That the vascular cords (midribs) of these inner semi-solid carpels occupy different positions in different genera. They may run horizontally outwards and then upwards in the ovary wall, like those of the outer whorl, or they may remain in the central column of tissue, or they may take up some intermediate position.

3. That the ovules are not borne on the re-entering separated margins of valve carpels, but on either side of the midrib of semi-solid carpels, hence the placentation is not truly axile.

4. That the two rows of ovules in one loculus are not borne by a single carpel, but belong respectively one to each of the semi-solid carpels bounding the loculus on either side.

5. That the prolongation in some genera of the stylar canal as an actual discontinuity of the tissues down to the very base of the ovary, as in *Tulipa sylvestris*, does not result from the separation of hypothetical surfaces which, although showing no sign of fusion, must be presumed to have previously coalesced; but is due solely to persistence of actual separate surfaces of carpels which, though they have come into contact, have not undergone fusion.

VI. GENERAL CONCLUSION.

It will be clear from the various considerations set forth in the present account that the conception of the polymorphic character of the carpel leads in many cases to a widely different interpretation of the known facts in regard to the gynoecium from that based on the current view of monomorphism. The evidence upon which these conclusions rest is furnished by cases selected from a wide range of families. They prove beyond all doubt that in the course of evolution certain well-defined carpel types have made their appearance and are to be found in all groups of flowering plants. Through recognition of these different types we obtain a rational explanation of many seemingly aberrant or meaningless anatomical features which now fall naturally into place and exhibit their true significance. In the past some of these features would have been classed as monstrosities, and as such would have been deemed undeserving of further consideration from the point of view of evolution. Even to-day it may still be worth while to recall de Candolle's final summing-up after discussing the difficulty of determining the morphological nature of the petaloid structures present in the flower of *Begonia*. He says: 'A monstrosity arising in a Begonia flower would tell us more than all the arguments, all the analogies, all the observations of organogeny; but this monstrosity is still to seek.' To this we might now add that where such are found they may prove to have a further value in that they may shed new light on the race history.

On the 'Squamulae Intravaginales' of the Alismataceae and Butomaceae.

BY

AGNES ARBER, M.A., D.Sc.

With eleven Figures in the Text.

(i) INTRODUCTION.

I N a paper published in this journal in 1923 [1] I discussed the little
squamules which occur among the leaf-bases of the Helobieae. At that
time I had been unable to trace the mode of origin of these structures in
the Alismataceae, so the question whether this family fell into line with the
rest of the cohort had to be left open. In the present note an attempt is
made to fill this lacuna by describing further observations on the Alis-
mataceae and the related Butomaceae.

(ii) OBSERVATIONS.

Alismataceae.

Sagittaria sagittifolia. L.

It will be seen from Fig. 1. which represents a single leaf-base of the
Arrowhead, *Sagittaria sagittifolia*, with its associated squamules, that these
structures are very numerous and delicate. The extreme crowding of the
leaf-bases is an additional factor which renders the task of tracing the origin
of the squamules more difficult than in many of the Helobieae. I have
found the relations of these structures easiest to understand from a study of
serial sections through young axillary buds. Figs. 2 A–C represent selected
sections from a series passing upwards from below through such a bud.
They are cut close to the base of the axillant leaf l_1—so near to its base,

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FIGS. 1, 2 A–C, 3 A, B. *Sagittaria sagittifolia*, L.. Origin of squamules, *sq.*, which are dotted throughout. Fig. 1. Transverse section of a sheathing leaf-base to show associated squamules (× 47); it is probable, though not certain, that all the squamules indicated belong to this leaf. Figs. 2 A–C. Transverse sections (× 193) from a series from below upwards through a bud in axil of leaf l_{+1}; the bud-axis, *b.ax.*, bears a prophyll, *pr.*, with median bundle, *v.b.pr.*, followed by leaf l'_2; *sq.l.*, squamules belonging to l_2 (i. e. the leaf borne by the main axis next above l_{+1}, the leaf axillant to the bud); *l-s.l.*, leaf-skin of l_2; *sq.pr.*, squamules borne by bud-prophyll. Fig. 3 A. Transverse section of centre of a small lateral bud to show origin of squamules from leaf *l.* (× 23). Fig. 3 B, the part of Fig. 3 A marked with a ×, more enlarged to show origin of squamules (× 193)

indeed, that it is fused with the axis except in the median region, where a small pocket is left for the bud. It will be seen that the bud bears a prophyll, *pr.*, and in Fig. 2 B a second leaf, l'_2, is visible, superposed to the prophyll. In the ' south ' part of the sections it is possible to distinguish two sets of squamules—*sq. pr.* and *sq. l.*₂. The squamules of the prophyll, *sq. pr.*, arise from its outer surface near the base ; their origin is demonstrated in all three figures, but perhaps most clearly in Fig. 2 C. The squamules *sq. l.*₂ are related to the leaf l_2 borne by the main axis. This leaf, which succeeds the axillant leaf l_1, has no free existence in these sections, but on the leaf-skin theory [1] the surface of the ' axis ', marked $l\text{-}s.l._2$ in Fig. 2 A, belongs to it. It will be seen that it is from this ' leaf-skin ' that the squamules *sq. l.*₂ arise. The squamule marked with a   in Fig. 2 C belongs to the *sq. l.*₂ group ; it touches the prophyll, but this is probably a case either of mere contact or of secondary connexion.

The mode of origin of the squamules in the ' north ' part of the sections is less easy to follow with certainty, but the history here is probably the same.

For confirmation. part of another axillary bud is represented in Figs. 3 A and B : it can be distinctly seen that the squamules associated with the leaf *l.* are budded off from its outer surface, at a level so close to the base of this leaf that it is only in part free from the axis.

Sagittaria natans, Michx.

In order to compare the behaviour of the squamules in another species, I cut sections of *S. natans*. Here, as is shown in Figs. 4 A and B, the squamules which occur inside the leaf l_1. really belong to the leaf-skin of the next leaf. l_2. which at this level has not begun to detach itself from the axis. *S. natans* is thus strictly comparable with *S. sagittifolia* as regards the origin of the squamules.

The Seedling of *Alisma Plantago*, L.

I have cut serial sections through several seedlings of the Water Plantain, *Alisma Plantago*, in order to trace the relation of the squamules to the first leaves. The basal region of the outer surface of the cotyledon, where, by analogy with the later leaves, we might expect to find squamules, passes over into the piliferous layer (*pil.* Fig. 6 A) without bearing any squamules. That the cotyledon should differ from the succeeding leaves in not producing squamules need not, however, surprise us, for a marked difference in epidermal character between the hypocotyl and seed-leaf on the one hand, and the plumule on the other, is by no means infrequent in seedlings.[2] The

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[1] Saunders, E. R. : The Leaf-skin Theory of the Stem. Ann. Bot., vol. xxxvi, 1922, pp. 135–65, thirty-four text-figures.

[2] e. g. Saunders, E. R., loc. cit., p. 136, Figs. 1 and 2.

first plumular leaf (l_1, Fig. 6 B), produces a pair of squamules ($sq.l_1$, Fig. 6 A) at its base, and the second plumular leaf (l_2, Fig. 6 B) bears a second pair ($sq.l_2$). I have not yet succeeded in determining at what stage in the development of the seedling the pair of squamules gives place to the larger number characteristic of the mature leaves.

Sagittaria natans (4)

Butomus umbellatus (5)

Alisma Plantago (6)
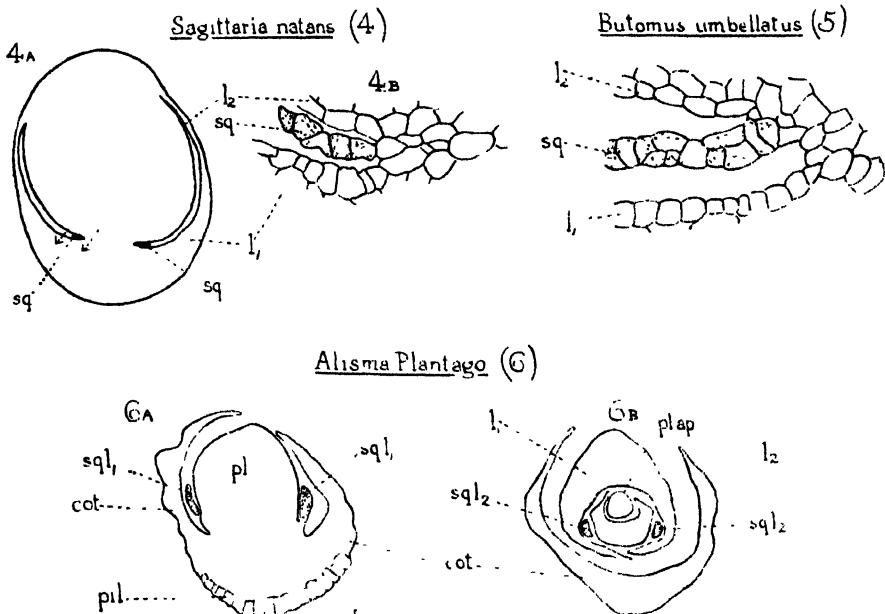
FIGS. 4 A, B, 5, 6 A, B.   Fig. 4. *Sagittaria natans*, Michx. ; Fig. 4 A, transverse section (× 47) of an axis bearing the leaf l_1, which is cut so near to its base that it is not completely detached. The squamules, *sq.*, belong to the leaf l_2, which has not begun to free itself.   Fig. 4 B, the part in Fig. 4 A between the arrows, more highly magnified (× 193) to show the origin of the squamule from the leaf-skin of l_2.   Fig. 5. *Butomus umbellatus*, L., part of transverse section through apical bud (× 193) to show a squamule arising from the leaf-skin of l_2, the leaf next above l_1.   Fig. 6, *Alisma Plantago*, L., two sections from a series passing upwards from below through the cotyledon and plumular bud of a seedling (× 47) ; *cot.*, cotyledon ; *pl.*, plumular bud ; *pl.ap.*, apex of plumule ; $sq.l_1$ and $sq.l_2$, squamules borne by leaf-skin of first and second plumular leaves, l_1 and l_2; *pil.*, piliferous layer, coming into view over part only of the surface, since its upper limit rises higher in the median region of the cotyledon.

Butomaceae.

Butomus umbellatus, L.

For comparison with the Alismataceae, I cut serial sections through a shoot-apex of the Flowering Rush, *Butomus umbellatus*. Here again I found that the squamules, which arise in the angles between each leaf-base and the one which succeeds it, definitely belong to the younger of the two leaves in question. In Fig. 5, for instance, the squamule *sq.*, lying between the leaves l_1 and l_2, is developed from the epidermis of the higher leaf l_2.

Hydrocleis Commersonii, Rich.

The mode of origin of the squamules in *Hydrocleis Commersonii* resembles that described for *Butomus umbellatus*.

(iii) CONCLUSIONS.

A study of the Alismataceae and Butomaceae has shown that these two families agree with the other Helobieae as regards the mode of origin of the 'squamulae intravaginales'. These structures are developed, not from the ventral epidermis of the leaf which immediately encloses them, but from the dorsal epidermis of the succeeding leaf, and should not, therefore, be described as ' axillary '.

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The Geographical Distribution and Ecology of Passerina.

• BY

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With four Figures in the Text.

PASSERINA, belonging to the Thymelaeaceae, is one of the most generally distributed genera of ericoid shrubs in the Cape Peninsula. As such it was the subject of some observations on the water relations of ericoid shrubs, published in 1921 (25). The experimental material was obtained from a clearing in the pinewoods on the outskirts of Capetown, where, within four or five years of the removal of the pines, native shrubs. notably species of *Rhus*, *Cliffortia*, and *Passerina*, had made considerable headway and covered a large part of the area with open vegetation to a height of some two to four feet. The work was begun in the very dry summer of February and March 1920. In the following spring, when the plants came into flower, it was found that two species of *Passerina* were present which till then had not been distinguished. Extended observation revealed that the two species generally selected soils of distinctive types. Their occurrence together in the first locality was exceptional, and due largely to the conjunction there of soils suited to each.

When other species were subsequently met with, the strictness with which each species was confined to a distinctive type of habitat, and the rarity of overlapping, were everywhere the outstanding feature of their occurrence. Generally each was a prominent. if not predominant. constituent of the vegetation where it occurred. These two facts together pointed to the genus as one which might provide a useful group of indicator plants, and in any case seemed of sufficient interest to warrant further study of its distribution.

The genus was, however, badly in need of revision. The two species first studied appeared not to have been previously recognized as distinct. Attempts to identify others were unsuccessful, and only served to show

that the classification of the genus was in a state of confusion. It was necessary to start practically *ab initio*, to study the morphological characters, distinguish the species, and establish the nomenclature on a sound basis before much progress could be made.

This task, though laborious, provided the opportunity of forming a first-hand impression of the morphological interrelationships of the species, unbiased by geographical considerations such as Willis's Age and Area theory might suggest, so that conclusions arrived at independently from the two sides might be compared. The orderly geographical distribution which finally revealed itself was nevertheless a welcome confirmation of the soundness of the classification.

Meanwhile, more extended field-work did nothing to weaken the impression that each species was adapted to a distinctive range of environmental conditions, and made it increasingly evident that no consideration of the geographical distribution of the species in its wider aspects could be satisfactory which did not take account of their ecological relations.

A further feature of special interest was that the distribution of the genus, which is endemic to southern Africa, corresponds in a striking manner with that of the 'Cape flora'. On the one hand, there is a marked concentration of species in the south-western region of winter rainfall. Six out of fifteen described species occur, moreover, in the Cape Peninsula and the adjoining sandy flats, a small area renowned for the richness of its flora. On the other hand, outlying species are found on the mountains of Little Namaqualand, or on the Drakensberg Range extending far to the north, while the genus is represented on almost all those higher mountain ranges of the south which, surrounded on all sides by karroid plains, retain towards their summits vegetation of typically south-western type. Such parallels suggested that an intensive study of this genus might throw light on some of the general problems presented by the Cape flora as a whole.

In the revision of the genus, already published (27), fifteen species have been described and the recorded localities detailed. In the present paper it is proposed to give an account of their mode of occurrence in parts of South Africa where somewhat intensive observation has been possible, to outline the geographical distribution of the species, as far as it has been recorded, and to consider the available data in their general bearings.

HABITATS AND RANGE OF THE SPECIES.

The species are all gregarious ericoid shrubs which rank as pioneers from the successional point of view. The opposite and decussate leaves are small and heath-like, mostly narrow and involute, forming a woolly

groove on the upper side to which the stomata are confined. In most species they are more or less erect, exposing the palisade assimilating tissue on the under side to the light, or are even adpressed, with the groove close against the pubescent or woolly stem. In some species the groove has been observed to close during prolonged drought (25), and in most species it closes when the water content is experimentally reduced. The flowers are wind-pollinated, with exposed mop-like stigmas and exserted stamens that produce an abundance of dusty pollen. Though individually rather inconspicuous they are usually numerous, clustered in short spikes at the tips of the branchlets, so that collectively they impart their reddish or yellowish colour to the whole shrub. Indeed, in the height of its brief flowering season the relative abundance of a species is often clearly displayed and its distribution can readily be followed.

Distribution in the Cape Peninsula.

The species that occur on the Cape Peninsula and the adjoining Cape Flats are *Passerina vulgaris*, Thod., *P. paleacea*, Wikstr., *P. rigida*, Wikstr , *P. ericoides*, Linn. (= *Chymococca empetroides*, Meisn.), *P. filiformis*, Linn., and *P. paludosa*, Thod.

P. vulgaris is the commonest and best known. It is very generally distributed on the lower slopes of the mountains and in parts of the Cape Flats on well-drained soil of coarse sand or gravel. It establishes itself readily from seed on open soil of this character.[1] Like the majority of the species, it grows best where fully exposed to the sun. In places somewhat sheltered from wind and undevastated by recent fires it grows robustly to a height of six or eight feet; while in the National Botanic Garden at Kirstenbosch there are lax specimens ten or eleven feet high between small pines and Hakeas above the Protea Garden. In partial shade it is less robust and often assumes a weeping or semi-weeping habit. It does not tolerate deep shade. In short, this species is a light-demanding pioneer of the Cape maquis on sandy or gravelly soil, notably poor in salts, such as is formed in abundance by the weathering of Table Mountain sandstone.

P. rigida, *P. ericoides*, and *P. paleacea* all grow on sand. Of these *P. rigida* is very local, occurring only on the floating dunes just east of Muizenberg, near the sea, as a robust shrub with stout straight stems two to four feet in height. Along with it *P. ericoides* also occurs with arcuate stems, seldom more than a couple of feet high, and shining scarlet berries. The latter is more abundant than *P. rigida*, and clearly more successful. It is common on exposed front dunes, also in many other parts of the coast of the Cape Peninsula.

In contrast with these two species *P. paleacea* is abundant and wide-

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[1] Cf. Marloth (15), p. 135, where it is referred to as *P. filiformis.*

spread on drift-sand away from the sea. Near Muizenberg, for example, immediately behind the dunes on which *P. rigida* and *P. ericoides* flourish, *P. paleacea* occurs only in a stunted form with dwarf or prostrate habit, but farther inland it grows erect to two or three feet in height, or even six feet if sheltered by other shrubs from the full force of the wind. It extends right across the sandy Cape Flats. It also occurs in abundance on the margins of the drift-sand that is blown up over the slopes of the mountains above Glencairn and Hout Bay. It is found on partially fixed rear dunes near Slangkop Point, and in other localities where similar conditions prevail.

The habitats of the remaining two species, *P. filiformis* and *P. paludosa*, are of very different types. *P. filiformis* is localized in the north-western part of the peninsula. It may be said to divide with *P. vulgaris* the lower north-western and western slopes of Table Mountain, the latter occupying soils dominated by Table Mountain sandstone, *P. filiformis* finer soils formed of weathered granite or Malmesbury clay, commonly in association with *Elytropappus rhinocerotis* (the Rhenoster bush, a common weed of derelict agricultural soils) and *Relhania genistifolia*. An instructive instance of the influence of soils in determining the distribution of the two species was observed on the sandstone boulder-strewn western slopes of Table Mountain above Camps Bay. *P. vulgaris* was common except for a stretch of some fifty yards, which received the drainage from a small farm where cattle and pigs were kraaled. Here *P. vulgaris* was practically absent, while *P. filiformis* was common, although it was not found elsewhere on this part of the slopes. *P. filiformis* also occurs at higher altitudes on the sandstone buttresses, where a soil richer in humus accumulates. Apart from one doubtful record, it has not been found on the Cape Flats nor anywhere on the eastern side of the mountain.

P. paludosa grows in marshy places and round the margins of vleis of a rather brackish character, where the soil dries out in summer. It is usually associated with *Dovea tectorum* (Restionaceae) in a well-marked zonal plant community. It is general over the more low-lying south-western part of the Cape Flats. *P. paleacea* and *P. paludosa* often grow near one another, *P. paleacea* on dunes or dry sand, *P. paludosa* in hollows which in winter are waterlogged or flooded; but they are separated by a zone of variable width in which neither species finds the conditions congenial.

Of these six species it thus appears that each has its own type of habitat, which can be defined largely in edaphic terms, and that only in the case of *P. rigida* and *P. ericoides* are the habitats of the same type. Apart from this coincidence, which is not complete, since *P. rigida* is confined to a single small locality, the habitats of the different species are distinctive.

Distribution in South Africa generally.

The Cape Peninsula species. Looking beyond the Cape Peninsula one finds that three of these six species are widely distributed, namely, *P. vulgaris*, the commonest, and *P. filiformis* and *P. rigida*, the two most restricted within the peninsula. Of the other three, *P. paludosa* is unknown elsewhere, *P. ericoides* and *P. paleacea* have a limited range to north and east

P. vulgaris is the commonest species of the south-western region   It

FIG. 1.   Maps showing range of the species of *Passerina*.   The crosses mark the localities of unidentified specimens.   The heavy line if completed would pass through Clanwilliam and Mossel Bay (p. 195).

extends northwards to Saldanha Bay, Hopefield, and Tulbagh (Fig. 3, p. 186), eastwards as far as East London, and there is an isolated record of it for Natal, where the Table Mountain sandstone outcrops along the coast. The soil on which it grows is nearly always recognizably of the same sandy type.   On the other hand, the climate is very different in different parts of its range, from the region of winter rains and dry summer on the west, through the forest region with evenly distributed rainfall, to the region of

summer rains on the east ; and from near the coast inland up to 5,000 ft. altitude near Graaff Reinet.

P. rigida, which is very local in the Cape Peninsula, extends along the coast eastwards to Natal, always on the coastal sand dunes. Eastwards from Mossel Bay it is one of the commonest plants of the floating dunes, and is the first shrub to colonize the sand, behind low fore-dunes fixed by the fleshy-leaved *Scaevola lobelia*. At Port St. Johns, Moss records it (MS. in herb. propr.) as on the seaward side of the dune scrub. In Natal, at Winklespruit, Isipingo, and Durban, it is one of the first shrubs, often the first, on the summit of, or just behind, the fore-dunes ; but here it has eastern subtropical rivals, and on the other hand it is not confined to the fore-dunes but is occasionally found in gaps in the tall dune scrub that covers the slopes of the large rear dunes.

Thus it is true of *P. rigida* as of *P. vulgaris* that its distribution is correlated with that of a particular type of habitat distinguishable by edaphic characters, whereas it extends through a wide range of climate, from warm temperate to subtropical, with rainfall varying both in amount and in incidence. How far north it extends along the east coast is unknown. That Durban is its limit is improbable. On the other hand, Muizenberg on False Bay is clearly its western limit. It has been searched for without result in other apparently suitable places on the peninsula coast, and also farther north. The abundance of *P. ericoides* indicates by contrast that *P. rigida* is not so thoroughly at home in the south-western climate.

P. ericoides is found not only on the False Bay coast, but also at many parts of the colder west coast of the peninsula, and farther north, near Milnerton, on the shores of Table Bay, and at Blaauwberg Strand. It has not been found in the neighbourhood of Saldanha Bay, where the dune vegetation is predominantly succulent. Probably the diminishing rainfall limits its northward migration. It is besides of limited eastward extent, the farthest record being from the dunes of Walker's Bay (between Hermanus and Danger Point). That the limit is really a limit to its migration determined by environmental conditions is suggested by the fact that the range of *P. paleacea* along the coast is closely coincident. This species occurs on the partly fixed rear dunes at Blaauwberg Strand, where *P. ericoides* occupies the crests of the front dunes. Similarly east of Hermanus both species occur. Neither has been collected farther east than this. On the other hand, the coast between Danger Point and Cape St. Blaize has still to be explored botanically. There is a gap here in the records of *P. rigida*. The actual limits for *P. ericoides* and *P. paleacea* may therefore lie somewhere farther east between these points. Neither, however, is known from Mossel Bay, where the railway meets the coast and collections have frequently been made. *P. rigida* is there already abundant

as the foremost dune-fixing shrub. It is probable, therefore, that both *P. ericoides* and *P. paleacea* are strictly south-western species, in the sense that both are confined to the region of cool wet winters and dry summers during which hot, dry, southerly and south-easterly winds are prevalent.

Of the remaining two peninsular species *P. paludosa* is not known from any other district. The vleis of the Cape Flats are habitats in some respects perhaps unique ; I do not know whether vleis closely comparable are to be found near the south coast in the Bredasdorp and Riversdale Divisions, but apart from this possibility the facts indicate *P. paludosa* as a species limited to a very restricted locality, by adaptation to a peculiar type of habitat, yet there abundant.

P. filiformis, like *P. rigida*, though local in the Cape Peninsula, has a wide range in South Africa as a whole, and while apparently sporadic in the south-west becomes more frequent in the eastern districts. In the Transkei, Pondoland, and Natal it ranges from near the coast up to several thousand feet on the great escarpment. On the Drakensberg in Natal, Bews records it as a constituent of the maquis of the talus slopes, which he regards as transitional to forest. In reference to a specimen sent to me for identification he has informed me that it occurred at the margin of dense close bush as a much-branched shrub about 10 ft. high, ranking as a pioneer shrub in the forest succession. The soil was there ' typical deep High Veld acid soil '. At Redlands, in the Knysna division, Keet found it ' mostly on stiff soils of good agricultural quality (Bokkeveld beds, &c.) '. This evidence. as far as it goes, tallies with the observations made in the Cape Peninsula in that the types of soil contrast sharply with those on which *P. vulgaris* occurs. Whether *P. filiformis* is to be regarded ecologically as a forest pioneer in the peninsula is a question of some difficulty. The areas there occupied by forest have been much reduced, chiefly as a result of intentional or accidental veld burning. Fire after fire breaks out on the mountain slopes during the summer, usually on Sundays or public holidays, and notwithstanding the efforts of the rangers the vegetation is kept at early stages in the succession. The isolated patches of forest that escape destruction in the shelter of the ravines or on the more remote southern slopes of Table Mountain mostly lack a transitional fringe, and therefore stand out conspicuously from the surrounding maquis and are commonly regarded as representing outliers of a distinct plant formation. Yet indications of transition are not altogether wanting, and it may be that if protected from fire certain types of maquis would ultimately develop into forest. *P. filiformis* and *P. vulgaris* are both pioneers of sclerophyllous scrub on their respective soils ; but the heavier soils selected by *P. filiformis* would perhaps prove more favourable to the rapid development of forest, whereas those colonized by *P. vulgaris* favour typical proteaceous types of maquis. *P. filiformis* is, besides, distinctly the less intolerant of shade.

The Cape Peninsula marks the limit of the range of the typical *P. filiformis*, and it grows less luxuriantly there than farther east, where the summer is the rainy season. North of the peninsula, however, between Saldanha Bay and the Great Berg River (map on p. 186), a *Passerina* occurs, which I have distinguished as *P. filiformis*, var. *glutinosa*. It differs in having glutinous leaves and flowers, and in certain less obvious morphological features. The habitat is remarkably different from that typical of *P. filiformis*. Leaving Hopefield, where *P. vulgaris* grows on sandy soil, with Restionaceae and Proteaceae, the railway to Saldanha Bay approaches the Berg River in a north-westerly direction and then turns westwards. Here the vegetation changes rather abruptly and becomes predominantly succulent. The most abundant constituents over wide areas are *Zygophyllum morgsana*, a shrub some three feet in height with bifoliolate succulent leaves, and *Euphorbia mauritanica*, with numerous slender, terete, succulent glaucous stems. The succulent vegetation is, however, interrupted at intervals by streaks and patches of a non-succulent community, marked by Restionaceae and microphyllous shrubs. At Langeenheid Station three plants were collected from such a patch as predominating constituents. They were *Thamnochortus dichotomus*, *Passerina filiformis*, var. *glutinosa*, and a species of *Struthiola* which comes near to *S. leptantha*, Bolus, though it does not agree completely with Bolus's description. Specimens of a *Passerina* collected some fifty miles farther north at Alexander's Hoek are also probably to be referred to *P. filiformis*, var. *glutinosa*. *Struthiola leptantha* has a recorded range from the Malmesbury Division northwards to Little Namaqualand. These two associates thus both appear to be western rather than south-western.

As regards the habitat it is possible to do little more at present than point to the problems presented by the prevailing succulence of the vegetation of this area and by the sharply limited islands of ' restionaceous heath '. The soil over the whole area is sandy, but possibly only shallow with the underlying granite near the surface. The non-succulent patches may perhaps mark hollows in the rock surface in which water collects during the winter. *P. vulgaris* occurs on the outskirts of this area, not only to the east at Hopefield, but also to the south-west on low hills near the eastern shore of Saldanha Bay. It seems highly probable, therefore, that it is edaphic factors which prevent this species from penetrating the area and help to account for the peculiar character of its vegetation. On the other hand, the severity and length of the summer drought increase rapidly in this direction, as Fig. 2 illustrates, and there may be local differences in this respect of effective magnitude. But whatever the master factors may prove to be, it is clear that *P. filiformis*, var. *glutinosa*, like the forms to which specific rank has been accorded, has its own distinctive habitat.

The forest region. It is curious that, although *P. filiformis* proper is

a constituent of forest fringes in the eastern part of its range, where it is most at home, and although it extends from Natal to the Cape Peninsula, yet in the region of uniformly distributed rainfall, where forest reaches its greatest development, this species is apparently rare, and another, *P. falcifolia*, C. H. Wright, is found as a forest pioneer. Between George and Knysna *P. falcifolia* occurs on the margins of forest and within the forest
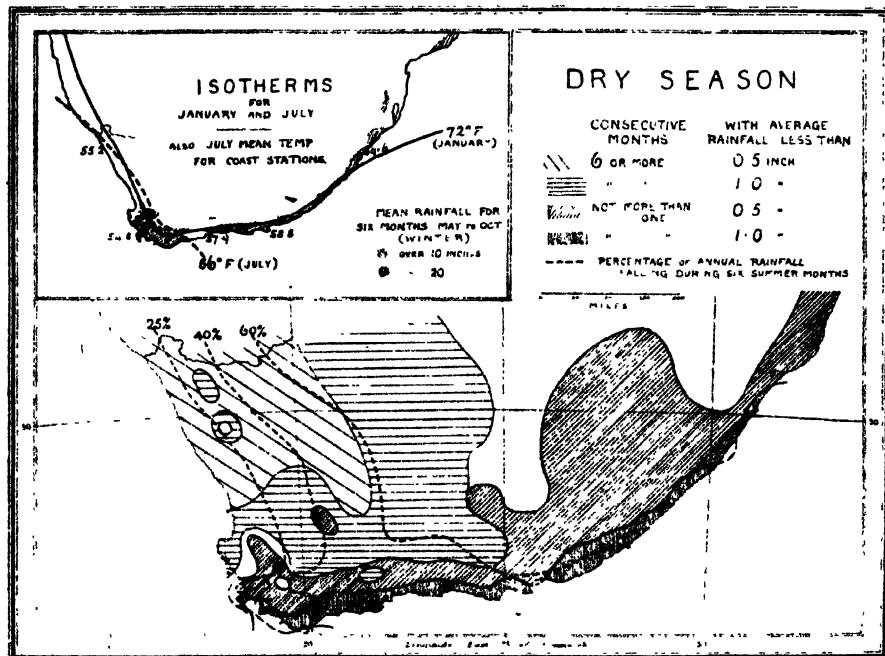
FIG. 2. Map to illustrate severity and incidence of drought, constructed on the basis of data given by Sutton (23). The broken lines giving percentage of annual rainfall during six summer months are taken from the Official Year-book of the Union (21).

Inset : isotherms and winter rainfall, to show that low winter temperature and winter rains distinguish the south-west Cape.

The two maps together illustrate the great variety of climatic conditions found within 100 miles of Capetown.

in more open places. In some parts it is abundant, and in favourable situations grows to a greater size than any other species of the genus. Sometimes it reaches a height of 15 to 20 ft., with a bole at least 3 in. in diameter, forming a small tree with light and graceful pendulous foliage. Here and there it extends on to the open veld between the forests, growing with a more erect habit to 6 or 7 ft. Its extreme range is from Mossel Bay on the west to near Uitenhage on the east, between the coast and the southernmost ranges of the folded belt. This is a relatively humid region without any marked dry season. Whether within this region its occurrence is determined to any extent by soil conditions is by no means clear. At

Knysna it grows on Table Mountain sandstone and apparently not on the conglomerate of Cretaceous age immediately in the vicinity of the town. In the Montagu Pass just north of George it is again on sandstone. But between George and Knysna the road traverses the Malmesbury beds (clay slates) for many miles, and *P. falcifolia* is conspicuously abundant. Even the sandstone soil is, however, modified by the forest vegetation, and its humus content, which is referred to by Marloth (15, p. 189), may be a factor of importance. The species has not been found on the sandy hills between the mountains and the coast, nor on the fixed drift-sand. of which there are large areas between George and Knysna. *P. vulgaris* is abundant there, but it nowhere encroaches on the preserves of *P. falcifolia*. Such mutual avoidance demands an explanation, yet climatic contrasts alone can hardly provide a satisfactory one. While *P. falcifolia* may require the moister conditions found near the mountains, these are not such as to be directly inimical to *P. vulgaris*. On the other hand, *P. vulgaris* does not colonize clay soils, while *P. falcifolia* can, and the humus nature of the forest soil, even on sandstone, may check the former even if it prove not to be essential to the success of the latter.

Other southern coast-belt species. Seven species have so far been dealt with, the six species of the Cape Peninsula and *P. falcifolia*. Of two other south-western Cape species there is little to say. *P. Burchellii*, Thod., is known only from a single locality, at the summit of the mountains above Genadendal (20 miles north of Caledon), where it was collected first by Burchell and again seventy years later by Bolus. It grew in crevices of the rocks, which are of Table Mountain sandstone, and formed many erect branches about a foot high from a thick woody base; but charred stumps of old branches afford clear evidence of veld fires.

P. Galpini, C. H. Wright, is known only from two localities, namely, near Riversdale, where Mr. E. E. Galpin collected the type specimen, and Mossel Bay. With regard to the former locality Mr. Galpin tells me that ' *P. Galpini* grows in fair abundance there in association with Ericaceae and Proteaceae, near the summit, on the ocean side, of a low range of hills from the top of which there is a steady descent to the ocean uninterrupted by any intervening hills. The soil is shallow and poor.' The rainfall is meagre even in winter (Fig. 2, inset).

Two species, *P. rubra*, C. H. Wright, and *P. pendula*, Eckl. and Zeyh., are definitely south-eastern in their distribution. *P. rubra* is apparently rather common around Port Elizabeth and Grahamstown. Of one locality near Grahamstown (Brak Kloof, Mrs. G. White, 1063) Prof. Schonland informs me that the veld is ' semi-karroid ' there. Zeyher (Z. 3779) records the species from calcareous soil, east of Port Elizabeth. This information, as far as it goes, points to finer or richer, not poor and sandy, soils as being favoured by it. Besides the two groups of localities around Port Elizabeth

and Grahamstown there are two other records. The most westerly, Muis-kraal, lies north-west of Riversdale, just north of Garcias Pass over the Langebergen, on the edge of the Little Karroo. The other, a recent record from the Langkloof at Keurbooms River, connects the last with the Port Elizabeth group, and it may be anticipated that further exploration will do something to bridge the gaps that remain.

P. pendula has a smaller recorded range. Zeyher collected it in Krakakama, south of Port Elizabeth, on sandy ground, and in the neigh-bourhood of Uitenhage, again in sandy places, near the Zwartkops River, as well as in the stony river bed. Recently specimens have been collected in the Langkloof. Thus the distribution of *P. pendula* runs parallel to that of *P. rubra*, but the available evidence points to contrasted types of soil.

These four species I have not seen growing. Of the remaining four, *P. glomerata*, Thunb., *P. obtusifolia*, Thod., *P. comosa*, C. H. Wright, and *P. montana*, Thod., I can again write from personal observation of their distribution in the field.

From Capetown to the Karroo. If the main line of railway ' up country ' is followed from Cape Town (Fig. 3), *P. vulgaris* is seen at frequent intervals for some fifty miles, often an abundant constituent of the vegetation. Beyond Wellington the line leaves the sandy alluvium of the upper valley of the Great Berg River for Malmesbury clay and cultivation, and *P. vulgaris* is also left behind. In waste ground the commonest plant is the Rhenoster bush, *Elytropappus rhinocerotis*. No *Passerina* is seen until, after leaving Porterville Road, the narrow pass to the Tulbagh Valley is approached. There, on slopes of sandstone to the south, *P. glomerata* grows in abundance. This species has been found by Prof. Compton a few miles south of this point on sandstone outcrops, but apparently does not extend far in that direction. It is abundant on the north-facing sandstone slopes of the pass. It is also conspicuous on crests and ridges capped by the relics of Table Mountain sandstone that occur here and there in the Tulbagh Valley over-lying Malmesbury clay. The clay soils, where not in active cultivation, are largely occupied by Rhenoster bush, and the patches of abundant *P. glomerata* with other characteristic plants of the sandstone flora, species of *Leucadendron*, *Erica*, &c., contrast, often very conspicuously, with the grey-green monotony of the Rhenoster veld.

The complementary distribution of *P. glomerata* and Rhenoster bush is reminiscent of the similar contrast between Rhenoster and *P. vulgaris* farther south. The latter species does occur in the Tulbagh Valley, but is only occasional and local there. In the Tulbagh Pass it only finds congenial conditions in the sandy soil of the roadside, while *P. glomerata* is evidently quite at home all over the rather steep sandstone slopes.

Leaving Tulbagh Road, the railway runs in a south-easterly direction, crosses a low watershed, and enters the valley of the Breede River.
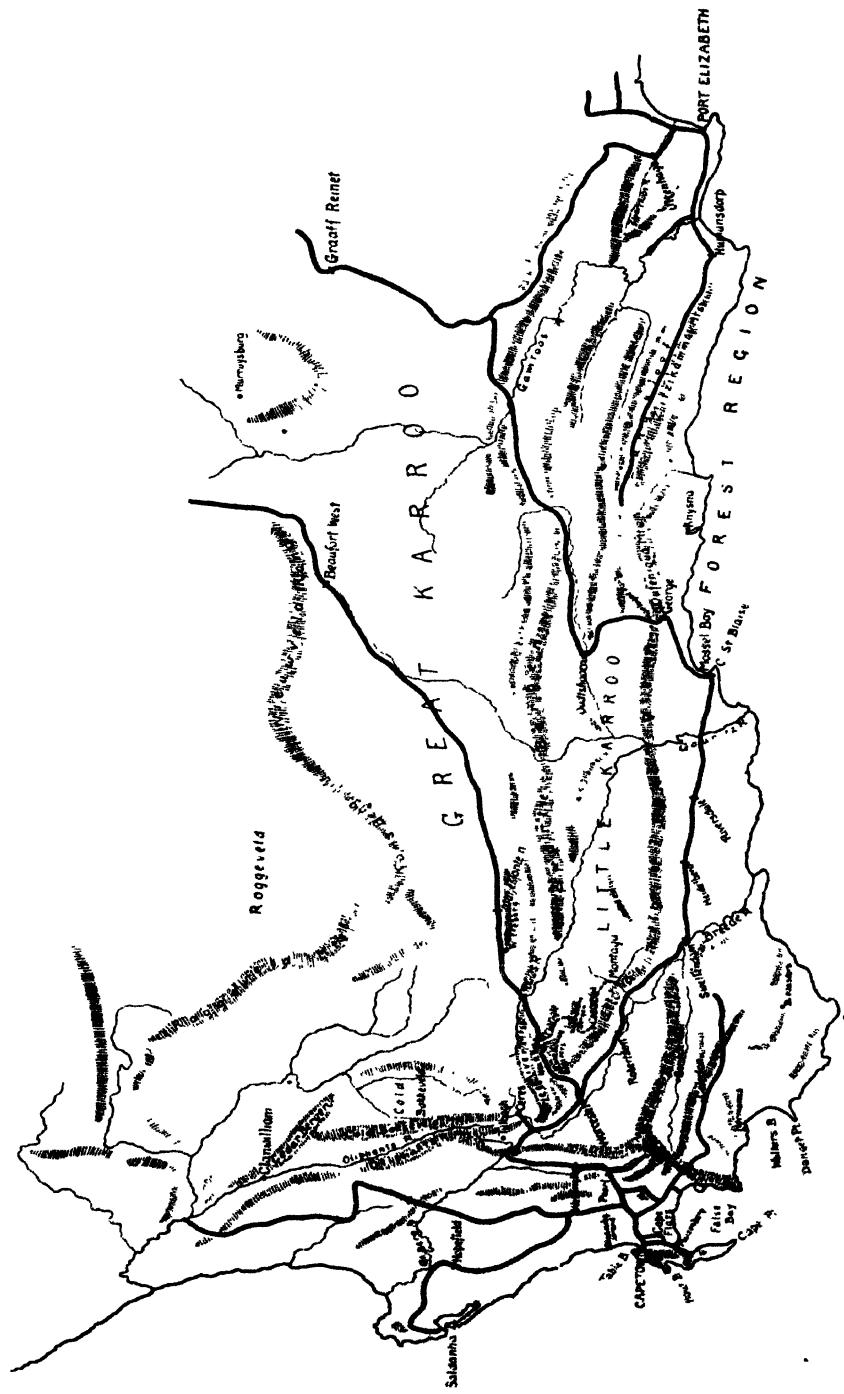
FIG. 3. Sketch-map of south and south-west Cape, showing the principal places and features referred to in the text.

P. glomerata is here apparently absent. The broad valley and the lower slopes are of Malmesbury clay; but on the sandy alluvium of the valley bottom *P. vulgaris* is locally abundant, depending probably on telluric water. Towards Worcester the railway runs along the north side of the valley over barren stony river gravels and level silty flats where no *Passerina* is to be seen.

At Worcester the line forks. The main 'up country' line enters the Hex River Valley through a narrow gorge. Here another species, *P. obtusifolia*, is conspicuously abundant on the isolated slopes of the Kwadouws Mountains, of hard reddish quartzitic sandstone, that bound the valley on the south side. When in flower it can readily be followed eastwards right along these slopes until the Table Mountain sandstone recedes at the head of the valley. At De Doorns it comes down on to patches of white sand and gravel that occur in the valley bottom.

Beyond De Doorns the Bokkeveld beds are reached. These are mostly shales, but are interspersed with thin beds of sandstone. The strata being nearly horizontal, characteristic flat-topped kopjes are formed, capped by the Bokkeveld sandstones. On the shaly slopes the flora is karroid, but on the sandstone summits karroid elements are accompanied by elements of a different type, among which *P. glomerata* is once more conspicuous. Thence onwards, at least to the neighbourhood of Matjesfontein on the western Karroo, this species is found locally common on outcrops of sandstone or quartzite—at Touws River on the summits of kopjes, where they are capped by Witteberg quartzite, also at Tweedside abundantly on similar quartzites on the slopes and summit of a mountain, accompanied by heaths, Proteas, &c., at Matjesfontein and Whitehill on the crests of ridges formed by outcrops of Dwyka sandstones, and on the crest of the Witteberg itself.

The ridges run parallel with the Witteberg Range, a massive outcrop of hard quartzites which has given its name to the Witteberg Series. Near the base of this main outcrop *P. obtusifolia* is found once more, but local. In one spot it was confined to a sloping patch of hard, bare. creviced quartzite facing north at an angle of some 40°. Here it was abundant, growing in the cracks of the rock, with a species of *Phylica* as its only rival in abundance. Around, but not encroaching, *P. glomerata* was fairly common. At another point *P. obtusifolia* was confined to more or less sheltered shelves on the steep sides of a rocky kloof. It is left for further exploration to reveal whether this occurs farther up on the mountains, and whether it follows the main escarpment farther to the east. In the case of *P. glomerata* at least, the eastern limit of distribution must be beyond Whitehill, for it is still locally abundant on the foot-hills there.

The other railway line from Worcester is the 'Garden Route' to Port Elizabeth, which runs at first through the Robertson Karroo over Uitenhage, Dwyka, and Bokkeveld beds, mostly conglomerates and shales, with a dis-

tinctly karroid flora.   Leaving the railway at Ashton, however, and penetrating the Langebergen by Cogman's Kloof, which leads towards Montagu, one again meets the genus.   On sandy soil, on the banks of the river, or by the roadside. *P. vulgaris* is found; while *P. obtusifolia* is frequently abundant on the hard quartzitic sandstone of the mountains. Only in one spot were these two species observed near together.   A narrow ridge of sandstone sloped down to a footpath.   *P. obtusifolia* extended along this ridge, growing in the crevices of the rock, while at the lower end small plants of *P. vulgaris* occupied little pockets of sandy soil.

As regards *P. obtusifolia* these observations probably justify the inference that it extends from Cogman's Kloof westwards along the Langebergen to the Hex River Valley.   It has been traced along the foot-hills on the north side of the Langebergen as far as Concordia, occurring sporadically, always on quartzitic crests.   Its characteristic habitat is hard reddish fissured quartzitic rocks fully exposed to the sun.   All the recorded localities are consistent with this generalization.   They include the Zwartebergen at Seven Weeks Poort, between the Great and the Little Karroo, the Winterhoek Range and the Zitzikama Range, and suggest that it follows the quartzitic ranges of the folded belt eastwards as far as the Port Elizabeth and Grahamstown districts, where the records indicate that it is not uncommon.   A specimen collected by Prof. Compton at Claypan Station, north-west of Clanwilliam, leads one to expect that in the northward direction it will be found to follow the mountains that flank the Oliphants River as far as the Table Mountain sandstone extends.

P. glomerata, on the other hand, is not confined, like *P. obtusifolia*, to the hardest quartzitic rocks.   It follows the foot-hills and lower slopes rather than the rocky mountains, even venturing in the western Karroo among plants the majority of which belong distinctively to the Karroo flora.   These facts, together with the recorded localities and the distribution of the geological formations, indicate that it probably follows the north side of the Hex River Mountains westwards and thence extends northwards through the Cold Bokkeveld and along the slopes of the Cedarbergen at least as far as Clanwilliam.

Little Namaqualand.   Similarly from the observed distribution in the neighbourhood of Tulbagh, along with other recorded localities, it may be inferred that *P. glomerata* follows the western slopes and foot-hills of the Oliphants River Mountains, northwards to the Konaquas Berg, where the Oliphants River turns westwards to the sea.   This is not, however, the limit of its range, for it was collected by Drège and by Pearson in Namaqualand.   There it is apparently a common constituent of those islands of 'Cape flora' which are found on the granite mountain masses of the Khamiesbergen and Vogel Klip.[1]   According to Pearson (18), 'On the

[1] Cf. Marloth (15), p. 292.

Khamiesberg and also at lower elevations to the north of Bowiesdorp, thunder rains occur more or less spasmodically in the summer season ', and Marloth distinguishes these areas as having a higher minimum annual rainfall than Namaqualand generally. Leliefontein, on the eastern slopes of the Khamiesberg, has a mean annual rainfall of 12·37 inches (23), whereas the surrounding plains have less than 5 inches (see also Fig. 2).

Between the Khamiesberg and the nearest record for the species to the south is a gap of a hundred miles. Pearson's descriptions of this region (16 and 17) leave little ground for supposing that *P. glomerata* occurs anywhere within it. The discontinuity must therefore be regarded as evidence of age, unless its small dry seeds, without special mechanism for dispersal by wind or birds, could have been transported across this distance.

Its southern limit is tolerably definite. A barren specimen collected by Mr. Stokoe on the Groot Drakenstein not far from French Hoek may belong to this species, but appears to represent only a sporadic occurrence. On the lower slopes and foot-hills it is not known to extend more than a few miles south of the Tulbagh Pass. Similarly on the western Karroo its limit is about 33½° south. On an expedition from Montagu (Fig. 3) westwards along the north side of the Langebergen to Concordia, and thence northwards to Triangle Station on the main railway line, *P. obtusifolia* was left behind in ascending the Wagenboomsbergen, and *P. glomerata* first met with some six miles to the north of that range, at a higher altitude.

On the same expedition another species, *P. comosa*, was found near Concordia on the valley flats, growing as a prominent constituent of a patch of Rhenoster veld in which the *Elytropappus rhinocerotis* was accompanied by *Relhania genistifolia*, as it so often is in the south-west at lower altitudes.

Like *P. glomerata*, *P. comosa* occurs in Little Namaqualand, where the type specimens were collected by Drège on the eastern side of the Khamiesberg. Here its most distinctive character, the hairiness of its leaves and bracts, is most strongly developed. Between these two localities, which mark the limits of its latitudinal range, the records are few, but probably do not adequately represent its distribution. A few specimens were found by Compton and Pillans on the summit of the Groot Tafelberg, at the head of the Hex River Pass, among an abundance of *P. glomerata*. Marloth records it from the western Karroo at Tweedside, on a quartzite mountain, and again on the Roggeveld, an elevated plateau within the great escarpment, where it was more abundant, and its situation corresponded more with that in which it was found in the Concordia Valley. The information is scanty ; but as far as it goes it suggests that this species, while occurring sporadically on sandstone and quartzite, is most at home on the finer soils formed by Bokkeveld and Karroo rocks in the valleys, and at altitudes of 3,000 ft. or more. Comparison of its distribution, as shown in Fig. 1, with the data

represented in Fig. 2, indicates that it has a higher moisture requirement than *P. glomerata.*

The Drakensberg. The last species, *P. montana,* is distinctive morphologically, ecologically, and geographically. Its inflorescence is sub-capitulate, and always strictly terminal, so far as has been observed, through the abortion of the apex. There are more records of this species than of any other, except *P. vulgaris,* and it has the widest range of all. It follows the great escarpment on the east, including the Drakensberg Range and its offshoots, from near King Williamstown to the Northern Transvaal, at altitudes from 3,000 to 10,000 ft. It is also recorded from Southern Rhodesia, near Umtali.

In his account of the ecology of the Drakensberg Range, Bews (1) describes this species (under the name *P. ericoides*) as a constituent of the patches of rocky scrub, with *Greyia Sutherlandi* commonly predominant, which occur on broken ground on the steep upper slopes of the mountains. At Van Reenen, where the railway crosses the border between Natal and the Free State, it grows on rocky summits and along the upper edge of the escarpments that bound the wooded ravines, at the margins of the *Leucosidea* scrub described by Bews. Thus the distribution of this species is probably well defined from an ecological point of view.

Its occurrence in Southern Rhodesia raises wider questions of plant geography, for the nearest locality to the south is distant some four or five hundred miles. The gap is occupied by the broad valleys of the Sabi and Limpopo Rivers. This low ground is skirted towards the interior by higher ground, along the Matopo Range and the north-eastern corner of Bechuanaland ; but collectors have frequently worked the Matopo Hills without recording *Passerina,* and the vegetation is of so different a type from that with which *P. ericoides* is associated on the Drakensberg that its occurrence there seems highly improbable.

As the ovoid seeds are from 1 to 2 mm. in diameter, their dispersal by wind across a gap of this size seems out of the question. Unless conveyed by migratory birds, or other occasional means of transport, the species must be old enough to antedate the breach in the escarpment by headward erosion.

GENERAL DISCUSSION.

The facts which have now been outlined show that the species of *Passerina* are distributed among various climatic regions and, within each region, among various types of habitat, in such a way as to demonstrate clearly that each species has a distinctive physiological constitution, which differentiates it from the rest as sharply as its morphological characters. In the face of the coincidences between range of species and range of

environmental conditions it is difficult to believe that the species are in general still spreading. The evidence points rather to limitation of range by climatic and edaphic factors. The edaphic influence is often remarkably clear, especially where two species grow near together, each abundant on its own type of substratum, yet neither encroaching on the domain of the other. There is no indication in such cases of any competition between the species.

Within this genus, therefore, the origin of species has involved both *morphological and physiological divergence in correlation with a divergence of habitat.* Whatever their mode of origin, it appears to be a necessary corollary that their specific constitutions are of survival value, even if the morphological characters that differentiate them are not. It would also seem to follow that they must have arisen within reach of the required types of habitat. Their existing distribution should, therefore, provide some clue to their places of origin.

Considering the spatial relations of the species we find that there is no single species known to be distributed over the whole range of the genus. If the genus arose from a parent stock of equal range this must have died out or had its range restricted, either by change of climate or by denudation.[1] Alternatively they may have evolved from a form of more limited range, itself therefore specialized in constitution, where it met new types of habitat and different climatic conditions. In either case the genus has extended the range of its physiological adjustability beyond that of the original stock.

The world-wide distribution of the Thymelaeaceae makes it a natural hypothesis that the ancestors of the genus migrated into South Africa from the north. If they had already developed the generic characteristics, ericoid habit and wind-pollinated flowers, they would probably have followed the mountains of the eastern escarpment which *P. montana* now occupies.

The claims of this species to be regarded as the ancestral species from which all the others are derived must, however, be discounted, because it has a specialized type of inflorescence peculiar to itself which, on morphological grounds, may be regarded as derived by reduction from the spicate inflorescence found in all the other species. As a modified derivative, place gives it no priority, for it might just as well have migrated northwards, along the track by which more remote ancestors, which had not evolved the generic characters, came south.

Failing *montana*, there is no species which, on geographical grounds alone, has any claim to be considered as ancestral. Apart from the barely

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[1] Gradual change *in situ* can hardly account for the degree of uniformity shown by the existing species throughout their present range, nor for the absence of intermediate forms between one species and another. This is so whether we assume direct action of the environment or selection from a widespread heterozygous stock. On the latter alternative cf. Hagedoorn (9), p. 228.

conceivable possibility of change *in situ* having occurred uniformly over a wide range,[1] the only alternative is to assume that each species has migrated from a definite locus of origin. If they have all been derived from one ancestral stock, their distribution should give a clue to the position of the original centre—the ancestral home. From this point of view the areas of convergence and concentration of species in the south suggest themselves as centres from which the more widely ranging species might have spread, and in which the generic stock might have originated. The fact that the genus is endemic to southern Africa, and the specialized features which characterize it. are both in keeping with a southern origin. The evolution of the ericoid type of leaf in *Thymelaea* is a parallel case in
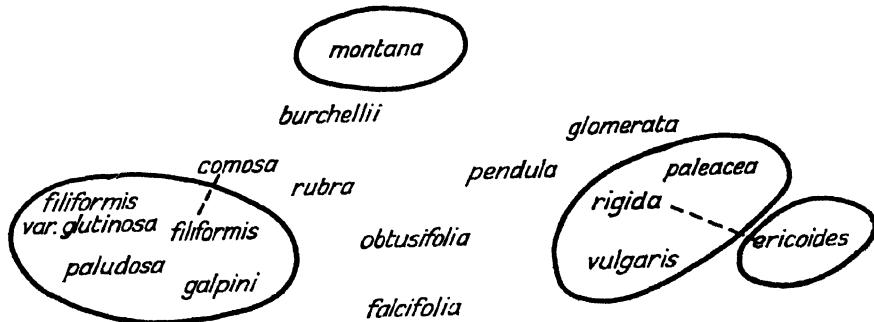
Fig. 4.

the Mediterranean region of the Northern Hemisphere, where similar climatic conditions prevail.

Morphological evidence. Before considering the facts of distribution further, it will be convenient to outline the evidence as to interrelationships of the species derived from a comparative study of their morphology.

The general impression gained is of a constellation of forms, no one of which can be singled out with any confidence as most primitive or closest to the hypothetical synthetic stock from which the genus might be supposed to have arisen.

The accompanying diagram (Fig. 4) is a tentative and somewhat crude representation of the constellation. It suffers from the obvious limitations imposed by confinement to two dimensions. It would be improved, for example, by rolling up, so as to bring *montana* and *falcifolia* nearer together. On the whole, however, it represents the degrees of similarity tolerably well.

In it two groups, each of three species, are distinguished, and two other species are fenced off separately.

Of the latter, *P. montana* has already been mentioned as peculiar in having a specialized inflorescence, which consists typically of four rather

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[1] For the genus as a whole it would be necessary to assume discontinuity as well as uniformity.

large bracts, with four axillary flowers, and is strictly terminal by abortion of the apex. In all the other species the inflorescence, whether small and few flowered, as in *glomerata* and *paleacea*, or lax and many flowered, as in *rubra*, is unmistakably spicate, and the apex resumes vegetative growth, at least on the leading branches.

P. ericoides was placed by Meisner in a separate genus, as *Chymococca empetroides*, on account of its shining scarlet berries, so different from the small dry fruit of most species, with membranous pericarp. The receptacle tube is also rather fleshy, and very early becomes barrel-shaped instead of remaining flask-shaped. The discovery that *P. rigida* has a fleshy coloured fruit has led to the reinstatement of *P. ericoides* within the genus, but it is nevertheless clearly divergent

The three species grouped together on the left of the diagram have the following characters in common : bracts with membranous glabrous wings which appear to be purely marginal expansions ; leaves bearded at the apex, at least in the bud (*P. galpini*) : and flowers having the receptacle tube quite glabrous within, and the sepals glabrous on the upper side. In *filiformis* and *paludosa* the outer sepals, as well as the leaves, are bearded behind the tip.

The group on the right in the diagram is distinguishable from all the other species by a peculiar anatomical feature, the marked development of hypodermal wandering fibres in the leaves, which has already been described for *vulgaris* (25). Outside this group similar fibres have been found only in one other species, *P. glomerata*, and in that they merely form a small tangled knot just behind the apex, which gives to the leaves their characteristic truncate or hump-backed profile.

Rigida and *paleacea* are so similar in the detail of their vegetative characters, small lanceolate leaves, with translucent keel in the upper part, adpressed to a thickly woolly stem, that barren specimens can in many instances only be distinguished with certainty by examining the distribution of wandering fibres, which differs slightly in the three species. The leaves of *vulgaris* are linear, usually longer, and not adpressed, and the young stems are pubescent. While the three species differ also in other ways, they appear to be derivable from a type which, in contrast with the *filiformis* group, had a receptacle tube and sepals more or less hairy on the inner side, leaves, bracts, and sepals quite glabrous on the back, and bracts hairy within and more or less leaf-like in texture. Each species has specialized features of its own, in addition to those common to the group.

The remaining species, occupying the middle of the diagram, unite in various ways features of both groups. *Pendula* and *glomerata*, like the *rigida* group, have leaves, bracts, and sepals quite glabrous on the back (as also have *montana* and *ericoides*). The others have leaves or sepals, or both, bearded or otherwise furnished with hairs on the back. All except *comosa*

have tube or sepals, or both, more or less hairy within. *Comosa* comes near *filiformis*, differing from it in the less specialized construction of the bracts as well as in the general and often conspicuous hairiness of its bracts and leaves.

Falcifolia, though superficially very like some forms of *filiformis*, has the receptacle tube finely hairy within and the sepals hairy above but glabrous below. The bracts, too, are closely woolly all over within, including the wings, which are hardly membranous and appear to be general rather than merely marginal expansions. The leaves bear a single line of hairs along the back.

Such forms as *falcifolia, comosa*, and *burchellii* lend support to the conception of a synthetic ancestral type, with hairy leaves, bracts similar to the leaves, but broadened by general expansion towards the base, flowers hairy within and without, and a dry fruit.

Further details will be found in the systematic account to which reference has already been made. Enough have been given to indicate the nature of the evidence on which the grouping of the species has been based, and from which it has been concluded—

(1) that two species, *montana* and *ericoides*, stand apart, as in their respective ways markedly divergent;

(2) that two groups of species are divergent in certain respects; and

(3) that the remainder combine features of the two divergent groups or lack their more specialized characters.

Correspondences between morphological and ecological relationships. In arriving at the conclusions just summarized regarding morphological relationships, every endeavour has been made to confine the judgement to morphological criteria and to avoid any bias from correspondences and relationships in habitat or geographical distribution. It is of all the greater interest, therefore, that the morphological grouping has brought together species with related types of habitat.

This is most evident in the *rigida* group. *Rigida, paleacca*, and *vulgaris*, and also *ericoides*, which is related to the rest of the genus through *rigida*, all grow on sandy soil—*rigida* and *ericoides* on sand dunes by the sea, *paleacea* on drift-sand behind the dunes, *vulgaris* on sandy soil or sandy gravel up on to the mountain slopes. On the other hand, *filiformis* and *comosa* have both been found as constituents of Rhenoster veld on soil of a very different type. The marshes, waterlogged in winter, that *paludosa* occupies differ less from Rhenoster veld, as far as soil conditions are concerned, than from the well-drained sands of the *rigida* group.

The scanty information available also indicates that *rubra* occupies soil approaching that of *filiformis* in type; *pendula*, on the other hand, sandy soil.

Regions of concentration. Comparing morphological relationships with

geographical distribution, we find that not only *montana*, but other widely ranging species, like *rigida*, *vulgaris*, and *filiformis*, are relatively specialized divergent derivatives of the ancestral stock. There is, however, no obvious correlation between range and morphological divergence, for other divergent species are restricted in range.

The derivative nature of widely ranging species leads back to a further consideration of the regions of concentration, which might be the regions where the ancestral stock gave rise to them.

The South-west Cape. The most important is the south-west part of the Cape Province—the Cape region in the narrower sense. If we take as an arbitrary limit a line drawn between Clanwilliam and Mossel Bay (Fig. 1), twelve of the fifteen species of *Passerina* have been recorded as occurring within it, namely, all but the last three in the following table, where the distribution of the species is summarized (see also Fig. 1).

Summary of the Distribution of the Species of Passerina.

*ericoides. **paleacea.* **paludosa* *galpini.*	Endemic to the south-west coast belt.
burchellii.	South-west endemic: isolated locality on southernmost range of folded belt.
glomerata. *comosa.*	Western species with southern limits just within south-west region.
filiformis var. *glutinosa.*	Endemic to southern part of western coast belt.
*filiformis. **†rigida.* **†vulgaris*	Coast-belt species ranging from Natal to the Cape Peninsula.
†rubra.	South-eastern species, just reaching the south-west region.
†obtusifolia.	Throughout the folded belt on the principal ranges, coming within the south-west region at the angle between the north-south and west-east folds.
†pendula.	South-eastern species of limited range from Port Elizabeth to Uniondale.
†falcifolia.	Endemic to the forest region of the south coast.
montana.	Eastern mountains, ranging north to Rhodesia.

* Occur in the Cape Peninsula and adjoining Cape Flats.
† Occur within thirty miles of Port Elizabeth.

Obviously *the concentration is complex.* It is due in part to endemism, in part to the convergence of western and south-eastern species which is correlated with geological and topographical features. The folded belt extends northwards as well as eastwards, and the western and southern folds meet in the south-west in a crumpled area of varied topography (Fig. 3). Ecological belts following the folds, both western and southern, and corresponding paths of migration converge in this region.

Within it a great diversity of conditions is found, both climatic and edaphic. The altitude varies from sea-level up to plateaus of over 2,000 ft. and mountains of 4,000–6,000 ft. elevation. A variety of geological formations

are exposed, and the annual rainfall varies from under 10 to more than 60 in. (cf. also Fig. 2). It is not surprising, therefore, that many species find congenial habitats there.

Of the endemic species of *Passerina*, *ericoides*, *paleacea*, *paludosa*, and *galpini* belong to the morphologically divergent groups and must be regarded as both ecologically and morphologically specialized. *Paludosa* and *galpini*, as well as *filiformis* var. *glutinosa*, are most closely related morphologically to *filiformis*; *ericoides* and *paleacea* to *rigida*. Both *filiformis* and *rigida* are eastern species reaching their western limits where the related endemics are found.

With *burchellii* the case is different. This endemic species is in many respects less specialized morphologically. It is a curious fact that some other forms which appear to have synthetic characters have also been recorded from isolated points on the southern ranges of the folded belt. They are represented only by single collections, and more thorough exploration of these ranges will be required before their relationships can be determined; but the facts as they stand suggest that we may have in these forms, together with *burchellii*, remnants of the old stock, a little modified in isolation, on mountains along which it originally migrated.

The western species, *glomerata* and *comosa*, which extend into Little Namaqualand, probably took their origin in the south and migrated northwards. Both present some specialized morphological features relating them respectively to the *rigida* and *filiformis* groups—hardly, however, as derivatives of the latter, so that their origin remains in some obscurity.

Vulgaris and *obtusifolia* are both southern species.

The South-east Cape. There is a second area of concentration in the south-east (cf. Fig. 1), but it is not quite so marked as the south-western. Eight species are found there (the last eight in the Summary on p. 195) including the three that do not occur in the south-west and five that do. Of the former, *montana* reaches its southern limit in the mountains of the King Williamstown division, *falcifolia* is endemic to the forest region, the eastern end of which is included in the area under consideration, while *pendula* is of limited westward range just to the north of the forest region.

Six of the eight species are found within a radius of 30 miles from Port Elizabeth, a small area which thus rivals the Cape Peninsula as far as *Passerina* is concerned.

This south-eastern concentration is clearly due principally to convergence. The area includes the eastern end of the folded belt and the southern spurs of the Drakensberg Range. It, too, provides a great diversity of edaphic and climatic conditions. The coast line here cuts obliquely across the folded belt so that a variety of geological formations are exposed in close proximity. This is particularly true of the Port Elizabeth–Uitenhage district, already mentioned as including six of the eight species. It is an

interesting peculiarity of this district that the rainfall shows two minima, one in summer and another in winter. At Port Elizabeth the summer minimum is the more marked of the two. In this respect the climate is comparable with that of the south-western region, but the summer drought is of short duration. The winter, too, is milder. The annual rainfall is rather low, 21 in. at Port Elizabeth, 17 in. at Uitenhage, but up to 30 in. and doubtless more among the neighbouring mountains. Other parts of the south-eastern area have a higher annual rainfall, with a maximum in summer.

The majority of the species of this region reach their maximum development in or near it. It is possible therefore to regard them as having originated there. All eight species could be so regarded, for even in the case of *vulgaris*, which is so abundant and widespread in the south-west, a south-eastern origin might perhaps help to explain its occurrence so far inland and at such high altitudes as 5,000–7,500 ft. on the Sneeuwbergen.

Past history of the genus. Some caution is necessary, however, in drawing inferences from existing distribution. Secular changes of climate or alterations in the configuration of the land due to erosion may have modified an earlier distribution, shifting the centres of greatest abundance or giving rise to discontinuities. Evidence is not wanting that the genus is old enough to have lived through such changes, in spite of the facts that it is endemic and is highly specialized in leaf and flower.

The Thymelaeaceae are a cosmopolitan family, predominantly Old World, and very well represented in Africa generally. As the American representatives are, with few exceptions, found in tropical South America and the West Indies. and we must go back at least to the middle of the Tertiary period for climatic conditions which would link tropical America with the tropics of the Old World, the family must date back so far at any rate.

As regards *Passerina* itself, the position allotted to it by Gilg in his classification of the family in Engler's ' Pflanzenfamilien ' (8) supports the view that it is of great age, though it gives little clue to its origin. Not only is the tribe Daphneae of world-wide distribution, but in the sub-tribe Passerininae are put, along with *Passerina*, the two Asiatic genera *Diarthron* and *Stellera* and the sub-tropical African genus *Dais*, with one species in Natal and one in Madagascar. It is doubtful whether this sub-tribe expresses the natural relationships of *Passerina*. There are very close general resemblances between it and certain microphyllous species of *Lachnaea*, another endemic Cape genus, which might indicate a closer relationship with the latter, and hence probably with *Gnidia*, which extends through tropical Africa to Ceylon and the Malay Archipelago. The morphological evidence is in any case sufficiently obscure to support the supposition that the origin of *Passerina* is remote in point of time.

The geological history of South Africa, moreover, reveals a land surface at least in large part as old as the Angiosperms. At the end of the Palaeozoic era, after the deposition of those sediments of the Karroo series that are characterized by the *Glossopteris* flora, the folding occurred in the south and west that brought into being the mountain ranges of the folded belt. The restriction of the area of deposition by these ranges led to the emergence of land over the Karroo region, and finally in Jurassic times occurred those tremendous disturbances that thrust up the Drakensberg volcanics. Since that time the sea has not encroached on the interior of the country. 'From the Cape ranges northwards to Central Africa the country has probably been exposed to the air since Triassic times' (21, p. 45).

The folded belt had been subject to much erosion before the sea encroached upon it during Cretaceous times, so that sandstone and quartzite had already been uncovered. After the late Cretaceous, a series of uplifts raised the general level of the whole country, and the land probably began to present topographical and geological features comparable in broad outlines with those of to-day quite early in the Tertiary period.

Erosion has, of course, continued to carve out river valleys and push back the great escarpment. The greater distance of this from the coast in the east than in the west indicates that the difference of rainfall between the two sides of the sub-continent is of long standing. But the evidences of past erosion in the west are such as cannot be explained on the basis of the present rainfall there. Marloth (15, pp. 377–8) has outlined the evidence given by Rogers, Passarge, and others of fluctuations of climate between rainy and dry, and particularly of a pluvial period perhaps more or less contemporaneous with the Pleistocene Ice Age of the Northern Hemisphere. Evidence from Tasmania of Pleistocene glaciation shows that the climate was colder at this time in the Southern Hemisphere also. Since then it has become progressively drier and warmer. According to Passarge the Kalahari during the pluvial period was probably in great part a series of huge swamps, like the Okavango of to-day. Conditions must, therefore, have been very different even in Namaqualand from what they are now. Marloth regards the islands of Cape flora on the mountains there as relics left from that period, when the Cape flora was far more widely distributed. We must also suppose that forest covered large areas on the mountain slopes in the south-west, where there are now only remnants in sheltered places.

Effects of secular changes of climate. Such discontinuities as have been noted in the distribution of some of the species of *Passerina* find thus a ready explanation. These species have been more and more restricted by gradual drying and warming of the climate. The discontinuities resulting from this restriction should be specially marked around the Great

Karroo. The existing records of *P. obtusifolia*, the species of the quartzite mountains, which represent it as concentrated in the west and in the south-east, with only detached and isolated localities on the mountain ranges between, may, therefore, give a true picture of its present distribution.

Similarly, *glomerata* and *comosa*, now confined to the west and north-west (*comosa* apparently to the least arid areas), under a moister climate would probably have found congenial conditions farther east than they now extend, in the neighbourhood of the great escarpment north of the Karroo. They may thus once have been connected with a place of origin in the south-east by links which have since been largely, if not completely destroyed. They cannot be confidently derived less remotely from any of the species with which they overlap in range to-day.

Age and area. As the change of climate has been in the direction of increasing warmth and dryness, those species have some claim to be regarded as older which are moisture-loving (*falcifolia*) or keep to the coolness and moisture of the mountain tops of the southern ranges (*burchellii*). Extended distribution with wide discontinuities is another criterion which would indicate *montana, glomerata* and *comosa, filiformis* and *comosa* as of considerable age.

On the other hand, neither continuity nor limitation of range is necessarily indicative of youth. For example, the more or less continuous distribution of *P. vulgaris* is correlated with the prevalence of the sandy soils which suit it. If its station on the Sneeuwberg Mountains is really isolated, we may have a significant discontinuity even in this case.[1] Limited range in the south-east (*rubra, pendula*) might be due to retreat from the drier west. Restriction to the south-west certainly points to origin in the south-west in the case of the more specialized species, but not necessarily to a very recent origin. *Burchellii* and other (unnamed) forms have already been referred to as possibly some of the most primitive, though extremely local.

It is evident that any correlation of age with range, such as Willis (28) has emphasized, is greatly complicated in this genus by the operation of other factors.

As further collecting may be expected to provide additional data it would be premature to speculate on the significance of the various details of distribution as at present known. It is to be hoped that exploration will throw light on two points in particular. One has reference to the forms still existing on that portion of the great escarpment that

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[1] Specimens collected by Drège on the Nieuweveld Mountains near Beaufort West may belong here, and if so provide still another isolated locality; but the authenticity of the duplicate I have seen requires confirmation. It is possible, however, that civilized man has extended the range of *P. vulgaris* within South Africa, for this species has been recorded from Australia as an introduced weed near Melbourne (27 *a*).

connects the east with the west, and forms the northern boundary of the Great Karroo, including the Nieuweveld Mountains, the Sneeuwbergen, &c.: do they provide links between the western species and the eastern, or are they other specialized derivatives? The other point concerns the isolated forms of the southern ranges: do they represent the ancient stock relatively little modified in its original haunts? On the Drakensberg, too, further search may be rewarded by new discoveries, for Dr. T. R. Sim collected a barren specimen in the Goodoo Pass, one of the approaches to the Mont aux Sources, which was almost certainly a *Passerina* with hairy leaves (cf. *comosa* and the *incertae*). Was this another relic of the old stock?

Origin of species. Regarding the origin of species in this genus the data naturally do not provide convincing evidence in favour of any particular theory. Mutations can easily be assumed, and some sort of phylogenetic tree might be devised accordingly. Origin by segregation after crossing would fit in with the concentration of species in those regions where the geological and topographical conditions favour the meeting of different forms. On the other hand, the marked correlation of distribution with environment makes it impossible to reject without consideration the view that the environment may have been a causal factor.

A. L. and A. C. Hagedoorn (9) have given a very reasonable presentation of the case for the origin of species by gradual purification of isolated groups of a heterozygous stock. They assume the stability of the genes which make up the genotype. They also assume that the 'total potential variability' of a stock is determined by the number of different kinds of genes included in it. As the number of surviving offspring is always a fraction of those produced, the potential variability will tend continually to diminish. It will be less in small groups than in the whole stock, and, if such groups are isolated from one another, they will tend to become pure for different sets of genes. If they occupy different ecological niches Natural Selection will accentuate the divergence between them and the elimination of genes will not be purely a matter of chance.

The species of *Passerina* do *occupy different ecological niches*. They are, moreover, isolated from one another not only by divergence of habitat. The species that grow near one another in the Cape Peninsula flower at different times. The flowering period being short, it is amply sufficient for one species to begin to flower a month later than its neighbour. Hybridization must occur very seldom, if at all. Cross-pollination may nevertheless have been possible at rare intervals, and it is just in the regions of concentration of the species, in the south-west and the south-east, that the conditions may have favoured the meeting of species and occasional hybridization.

Although the relationships of the south-western endemics to the two wides, *rigida* and *filiformis*, at first sight support the theory of linear deri-

vation by mutation, it is nevertheless conceivable that the endemics might all have arisen as segregates from crosses between these two species and either the common and widespread *vulgaris* or other species now extinct or very local.

On general grounds, however, this view of the origin of species, which in essence is that of Lotsy (13), does not appear to be sufficient if it stands alone. By hypothesis the 'total potential variability' within a group of interbreeding forms tends always to diminish. It is only increased by crossing with another group. But crossing is only possible within a limited circle of allied groups, and the total potential variability of each such circle must tend to diminish. Geographical isolation subdivides these circles into smaller circles with still less potential variability. Is there then no process by which this progressive limitation of possibilities is transcended ?

Mutation induced by change of environment might be such a process. It is at least a remarkable coincidence that the two species of *Passerina*, *P. cricoides* and *P. rigida*, found on the seaward dunes should be the only species with fleshy fruits. Taken along with the frequency of succulence among halophytes generally, it is difficult to believe that the constitution of these species has not been influenced by the environmental complex. If *cricoides* has been derived in some such way from *rigida*, it is interesting to see how the increased succulence has invaded the whole shoot system. Not only is the pericarp completely succulent. but the receptacle tube becomes distinctly fleshy and larger than in other species. The leaves have lost the wandering fibres characteristic of *rigida*, and, further, the normal fibres associated with the bundles are only feebly developed ; while the inner walls of the epidermal cells are thick and mucilaginous, a feature which is much less uniform and marked in the adult leaves of *rigida*.

Filiformis var. *glutinosa* might be interpreted similarly as the result of physiological mutation conditioned by the environment. the correlated morphological changes being in this case comparatively insignificant.

The facts described in this paper certainly warrant emphasis of the physiological constitution as of more fundamental importance than the morphological features that the systematist has to use. It may be that a change of environment acting on the physiological constitution of an organism can sometimes bring about a permanent change in that constitution of a discontinuous and irreversible nature. Morphological changes of varying degrees of prominence, and not in themselves necessarily of an adaptive character, would generally be correlated with the physiological change.

It would indeed be surprising if the delicately balanced structure of the protoplasm were not susceptible of change of this kind under an influence straining the adjustability of the species, and persisting from generation to generation. It might not necessarily be a change in the chemical composition of the genes but in their colloidal structure—from one grouping of the

substances composing them to another more stable under the new conditions [1]—such changes of structure as may be reflected in the specific forms of starch grains (19 and 20). The allotropic forms of carbon and sulphur provide analogies of a lower order of complexity.

Endemism and climate. The large proportion of species endemic to the south-west suggests that the climatic conditions there are peculiarly apt to induce mutation. It is a striking fact that the south-western part of Western Australia is just such another region rich in species, many of them endemic, and has a similar climate.

Sir J. D. Hooker in 1859 concluded from his unrivalled knowledge of the floras of the world that ' It is in those tracts that have the most broken surface, varied composition of rocks, excessive climate (within the limits of vegetable endurance), and abundance of light, that the most species are found, as in South Africa, many parts of Brazil and the Andes, Southern France, Asia Minor, Spain, Algeria, Japan and Australia' (11, p. xiv). The ' Mediterranean climate' is remarkably well represented in this list.

The direct influence of climate on the mutability of species is not, however, the only factor that may have contributed to the richness and endemism of the Cape flora. The *climatic conditions hinder the establishment of completely closed communities*, especially on the sandy soils which are so prevalent. Grasses hardly ever form a turf. Broad-leaved plants casting deep shade are mostly confined to sheltered ravines. The foliage is predominantly of a light and open type which *allows the light to penetrate* between the narrow or vertically placed leaves (26). New forms would find many open spots in which to establish themselves, without meeting severe competition for light, provided that they could withstand the summer drought and found congenial soil conditions. Hence the mutability of the species, whatever its kind or degree, would tend to be more fully registered than in regions favourable to exclusive closed communities.

Bews (4) has suggested somewhat similar considerations regarding the flora of mountain ranges. These, he points out, are regions of unstable topography and variable climatic conditions. Judging from the number of endemic species, such variable and unstable conditions are favourable for the production of new species. Here the instability keeps the vegetation open, so favouring the establishment of new forms. The diversity of the environment will also influence the number of different species that can survive,

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[1] A. L. and A. C. Hagedoorn, regarding the genes as stable autocatalytic entities, suggest that different genes may be influenced differentially as to rate of increase by changes of environment, but retain their qualitative stability. It is conceivable, however, that quantitative changes of this kind might proceed far enough to bring about a readjustment with far-reaching consequences, involving even a qualitative change in the genotype itself. As a corrective to the rigidity of the ' biomechanical' view reference may be made to Gates, Mutations and Evolution (7 *a*), and McLean's Remarks on the Nature and Definition of Species (14).

since the less uniform it is the greater will be the number of different varieties of habitat.

Jaccard (12) has drawn attention to the influence of variety of habitat on the relative richness of floras, as indicated by the average number of species in a genus. He uses the reciprocal expressed as a percentage, which he calls the 'generic coefficient'. From his statistical studies of alpine and subalpine vegetation he has drawn the conclusion that the more varied the environmental conditions the smaller the generic coefficient, i. e. the larger the genera on the average. The generic coefficient falls, moreover, in an area of apparently uniform facies, as the size of the area considered increases, a fact which Jaccard explains on the same basis as due to the increasing number of varieties of habitat included. Harshberger (10) has given similar evidence on a broader scale for American vegetation.

From this point of view the south-western region of the Cape and south-west Australia are both remarkable.

The smallest generic coefficient given by Harshberger is 23 per cent. for the south-eastern United States, a very large area. For the mountainous State of Colorado, with an area of over 100,000 square miles, the figure is 24·1 per cent.; for Great Britain, with about 90,000 square miles, 24 per cent.; for Switzerland, with 16,000 square miles, 27 per cent.

Compare with these the estimates given by Hooker (11) as long ago as 1859 for south-western Australia, about 17 per cent. For south-eastern Australia the corresponding estimate is 25 per cent.; for Tasmania alone (22,633 square miles), 37 per cent.

The figures for South Africa are equally striking. For the south-west as a whole no comparable figures are available; but Bolus and Wolley-Dod's list for the Cape Peninsula (6) published in 1903 gives a coefficient of 23 per cent., although this area is only 197 square miles, and there can be no doubt whatever that the figure for the south-west Cape would be very much lower and rival Hooker's estimate for south-western Australia.

As in Australia so in South Africa the eastern flora has a higher generic coefficient, i. e. the genera are on the average smaller. J. M. Wood's 'Revised List of the Flora of Natal' (29) compiled in 1908 gives a coefficient of 26·1 for an area of 21,000 square miles. Bews's recent 'Flora of Natal and Zululand' (3) brings this figure down to 23·8 per cent., illustrating how increasing knowledge of a flora tends to lower the coefficient. On the other hand, Schonland's recent list (1919) for the Divisions of Uitenhage and Port Elizabeth (22), a district already referred to as a region of concentration in the case of *Passerina*, gives the relatively high coefficient of 32·4 per cent. for an area of 2,500 square miles. The number of species is of the same order as the number found in the Cape Peninsula, but the area is much larger and the species are distributed among nearly 50 per cent. more genera. To some extent this larger number of genera is doubtless due to

the fact that the south-east is the meeting ground of different floras, the temperate Cape flora and the sub-tropical eastern flora. Hooker's figures suggest a similar view of the south-eastern flora of Australia. Overlapping rather than endemism appears to be characteristic of these regions.

A low generic coefficient combined with a high degree of endemism must signify either unusually rapid production of new species or a long continuance of the process. For an endemic species is not only, in general, of local origin (unless a relic), but it has lacked either time or power and opportunity to migrate. *Passerina* indicates how important a factor confinement by specialization to particular types of environment might be in preserving the endemic character of a flora. In so far as the endemic species are confined they will remain endemic, and will accumulate as such. This seems a more probable explanation of a high degree of endemism in an old flora like that of the Cape than the view that the endemics are young and have not yet had time to spread.[1]

Clearly their specialization may not only have confined the endemics, but also have preserved them from invasion. This is probably true of the Cape flora as a whole. By reason of its specialization it is limited to the area which it at present occupies. But the distinctive climate to which it is so well adapted has guarded it against wholesale aggression from the sub-tropical and tropical floras to the north.

The salient climatic factors, as suggested in Fig. 2, are a relatively low winter temperature and a dry summer season. The prevalence of sandstone is an edaphic factor of considerable importance, for the Cape flora is in very large part a flora of sandy soils.

Plant growth occurs especially in the spring, when the temperature begins to rise after the cold wet winter, while the dry hot summer season is a resting period for most plants. Where the rainfall is sufficient the Cape flora holds its own against the arid floras of the Karroo and Namaqualand, while the summer drought and the low temperature during the growing season are an effective barrier against the subtropical and high veld forests and grasslands, closed communities, which predominate to the east and north-east.

The geographical position of the Cape flora has, however, protected it in a way which may be even more significant. It has not only been sheltered by the tropical belt from the vast reservoir of temperate forms in the Northern Hemisphere, for which the only general avenue of approach has been the narrow way of the mountains, but there has been *no cold temperate flora to the south* to subject it to devastating invasion during cold periods. Accessions to the flora have necessarily either arisen locally or come from the north.

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[1] Willis has emphasized the time factor; but I am inclined to think power and opportunity have been more effective in South Africa.

Thiselton-Dyer's hypothesis of culs-de-sac (7) starts from the predominant southward trend of migration.   He apparently pictures the southern ends of the continents of the Southern Hemisphere as traps into which waves of migration have driven a multitude of species which have become congested there.   He says: ' It cannot be doubted that slow but persistent terrestrial migration has played an enormous part in bringing about existing plant distribution, or that climatic changes would intensify the effect because they would force the abandonment of a former area and the occupation of a new one ' (7, p. 309).   It would be more correct to say that climatic change would obliterate from a former area and make migration into a new one possible.   Plants cannot be driven like sheep into a pen. The barrier of the southern seas has stopped further southward migration, but has not as such brought about any accumulation, nor has it interfered with the process of Natural Selection.

In so far as accumulation correctly described the process by which the present richness of these southern floras has been reached, it has been *permitted* by the particular climatic conditions that prevail.   That the peninsulas of the Mediterranean region, with a similar climate, also show a congestion of species [1] seems more than a mere coincidence.   But these also have been asylums where some degree of immunity has been preserved from competition with the aggressive vegetation of the cooler zones to the north.

In referring to the high degree of specialization of the southern floras both Wallace and Thiselton-Dyer have remarked on ' the little power the species possess of holding their own in competition or in adaptation to new conditions '.[2]   It may be questioned whether this is so unique a feature of these floras.   At any rate there is little evidence that other floras are able to transcend a certain range of climatic conditions.[3]   In South Africa the plants that appear aggressive in competition with the native flora come from similar climates—like the Hakeas and wattles of Australia and *Pinus maritima* from the Mediterranean.

In *Passerina* we have a genus which has shown adaptability in the past, for some of the most widely distributed and abundant species are, if we have read their story aright, derivatives from an old stock, itself specialized to a particular complex of environmental conditions. which have arisen in relation to new sets of conditions to which they are also distinctively specialized.   The process has not involved merely a subdivision of the original range between differentiating forms themselves progressively more narrowly specialized.   The former limits have been overpassed.

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[1] Cf. Darwin, More Letters, i, p. 453, where the congestion of species in the peninsulas of the Old World is held to point to long-continued southward migration.   Sicily, 2,549 spp. in 9,860 sq. miles—generic coefficient 24 per cent.

[2] Dyer (7), p. 309.   Cf. also Wallace, Island Life, pp. 527–8.

[3] Cf. on this point Willis (28), chapter iii and the literature there quoted.

At the same time the average range of adjustability of the species in a flora which has been relatively immune from wholesale invasion may well be less than in the Northern Hemisphere, where the most highly specialized and confined species would have been the first to succumb during the Pleistocene migrations.

In conclusion the desirability of intensive study of other Cape genera may be urged.

Passerina is not alone in showing a marked correlation between habitats and species. Species of *Phylica* are often associated with those of *Passerina* and in some cases appear to be more localized. For example, *Phylica rigidifolia* occurs with *Passerina glomerata* on the kopjes at Touws River, another species along with *P. obtusifolia* near Matjesfontein. *Phylica* would be well worth study, for it has about sixty species in the south-west Cape, most of them endemic, and sixteen in the Cape Peninsula alone. This is, however, another difficult genus in need of revision, especially as regards the many species with ericoid leaves and small flowers. Genera like *Erica* and *Pelargonium*[1] represent a further step in the direction of complexity. Such large genera may well illustrate the influence of additional factors in the problem of endemism, but would give some answer to the question how far physiological and ecological specialization can be carried. If, too, the past history of the Cape flora is ever to be reconstructed, the statistical methods which have served to demonstrate its unique features must be supplemented by intensive study of its characteristic genera.

SUMMARY.

The distribution of the species of *Passerina* indicates that each has its distinctive physiological constitution and is specialized to a definite range of environmental factors.

The genus is endemic and of considerable age. The species of greatest range are not the oldest, but rather, judging by morphological criteria, are derivative, while those with most claim to be considered synthetic and nearer the ancestral type are restricted in distribution.

The convergence and concentration of species indicates the south, and perhaps more particularly the south-east and south-west, as centres of origin.

The specialized endemic species of the south-west point to the endemism of the Cape flora as being correlated with the peculiar climatic conditions there, which (1) may directly favour mutability, (2) owing to the semi-open

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[1] Bews (2), in a discussion of the distribution of plants in South Africa, refers to the concentration of *Pelargonium* spp. in the south-west Cape (p. 16) and of *Erica* spp. in the Cape Peninsula and on the Van Staden Mountains (p. 22) near Port Elizabeth (cf. *Passerina*). Many other interesting facts are quoted, and the whole discussion is full of suggestiveness.

character of the vegetation may register that mutability more fully by allowing a larger proportion of the forms that arise to establish themselves.

The importance of confinement by specialization and of protection from wholesale invasion in preserving the endemic character of a flora are emphasized, as illustrated by the Cape flora as a whole.

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ADDENDUM.

Since the above was written another paper on questions relating to plant distribution in South Africa, by Prof. S. Schonland (On the Theory of 'Age and Area', Ann. Bot, xxxviii, p. 453, 1924), has come to hand. He too emphasizes the complexity of the subject. His analysis of the case of *Erica* (and its allies), by which he illustrates this complexity, lends support to the contention of our last paragraph that there is need for intensive study of such genera—a study in which comparative autecology and comparative morphology must go hand in hand.

NOTES.

AN OLD RECORD OF THE OVULE OF LARIX.—Some time ago the writer published an account of the morphology of *Larix leptolepis* (Proc. Roy. Dub Soc., xv. 28, 1918). The close relationship between *Larix* and *Pseudotsuga* in most points except habit was there brought out, but particular emphasis was laid on the ovule of *Larix*. Lawson (Ann. Bot., 1909) had already described the pollen-catching device and stigmatic outgrowths of the micropilar tube of *Pseudotsuga* and the phenomenon of its pollen germinating while suspended at the top of the tube, the pollen-tube growing in mid-air till it reached the nucellus. The writer described for *Larix* an ovular device to all intents the same as that in *Pseudotsuga*, with the difference that the pollen, after a period of attachment to the stigmatic outgrowths fell finally upon the nucellus, where pollen-tube development began. At the time of publication there seemed no record even suggestive of this ovular condition in *Larix*, at any rate in the later literature. Lawson thought *Pseudotsuga* unique ; Lotzy ('Bot. Stammesgeschichte ') regrets the absolute lack of knowledge of the reproduction of *Larix* : Coulter and Chamberlain are silent, and so on. But there has just been brought to the writer's notice an old article—N. Géléznoff, ' Sur l'embryologie du Mélèze ', Bull. Soc. Nat. Moscou, iv. 22, 1849—which seems to have been entirely overlooked. Mr. A. Henry, Professor of Forestry, Dublin, having purchased, while on the Continent, a number of old pamphlets on the Conifers, discovered this and, knowing the writer's interest in *Larix*, passed it over to this department. Here appears quite a full account of the reproductive morphology of *Larix*. Of course, remembering the date, when ideas of cell-formation and fertilization were still so uncertain, there is much strange reading. But the essential facts are there, and, among other points, the stigmatic development of the ovule, the incurving of the edges of it and the subsequent deposition of the pollen-grains on the nucellus are fully described.

    It seemed remarkable that so complete an account could have been overlooked, and a search was made in some of the older literature especially to see if any further reference appeared to the stigmatic apparatus of *Larix* prior to Lawson's discovery in the Douglas Fir. In only one place did any reference appear, namely, in Strasburger's 'Die Angiospermen und die Gymnospermen ', 1879. Here—Pl. X, Fig. 38 ; Pl. XI, Fig. 32—drawings of the ovule of *Larix europaea* can be seen, with pollen adhering to a one-sided prolongation of the integument. In the script, however, he only says (p. 88), ' Die Anlage des Integuments ist zweilippig, doch sind beide Lippen zunächst fast in gleicher Höhe, dann die randständige sogar höher als die innenständige inserirt ; die innenständige ist es, die hier allein zu einem starken, freien Lappen anwächst '. He was probably unacquainted with Géléznoff's paper of thirty

years earlier, while Lawson appears to have overlooked the interest of Strasburger's drawings when deeming *Pseudotsuga* unique. Strasburger's drawings of *L. europaea* show a one-sided stigmatic development of the integument—the other side being no higher than the nucellus even when the embryo sac was already well formed. Géléznoff does not name his species—it was probably *L. sibirica*—but he too describes a one-sided stigmatic development. In this, these two species differ from *L. leptolepis*, in which almost the whole of the micropilar mouth is involved just as in *Pseudotsuga Douglasii*. The writer hopes in the spring to make a more complete examination of the ovules of as many species of *Larix* as possible. But even though the point be not a great one, yet it seems only just, in view of this peculiar pollination device of *Larix* and *Pseudotsuga*, that to the long dead Géléznoff should be restored full credit for having first described it.

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VITALITY OF DORMANT BUDS.—A remarkable case of the persistence of vitality in the dormant buds of a severed branch of an old Mulberry tree was recently brought to the notice of the writer by Mr. Herbert Leaf in his garden at The Green, Marlborough.

In this old-world garden there is a fine old Mulberry tree, which, forty-eight years ago, was beginning to shed a few small branches in proof no doubt of its mature age at that date (1876).

More than once since then branches have been removed to relieve the old tree of undue weight.

On October 10, 1907, a large portion of the tree was blown down in a storm and most of the branches were burnt, but one branch about five feet in length and twenty inches in circumference was put aside, standing upright, in an old dry stable.

In July 1913 it became necessary to prop up a big branch of the old tree, and Mr. Leaf, remembering the log put away in the stable and finding it to be the right height for his purpose, used it as a prop.

The prop which had been standing in the stable for nearly six years was sunk about eighteen inches in the ground, and in the following spring some signs of returning life were noticed near the ground level and a dormant bud developed into a shoot some six inches above the ground.

Now the middle portion of the prop is bare of bark, but a strip remains near the shoot. During the last ten years the shoot has grown slowly and is now a very healthy growth with large leaves and measures some two feet in length, and is about three-quarters of an inch in diameter. The base of the shoot is thickened and corky and appears to be firmly attached to the woody portion of the prop (*vide* figure).

It is of course well known that in the bark of trees there may be large numbers of dormant buds, some of which originally were formed in the axils of leaves borne on the trunk and branches when they were quite young. When, for instance, a large limb

Mulberry tree and prop.

breaks off from a tree, numbers of these dormant buds in the main stem frequently develop into leafy shoots after lying dormant for many years (see Marshall Ward, 'Trees', vol. v, p. 56).

The case of Mr. Leaf's Mulberry branch, however, is remarkable and interesting, the branch having been severed from the parent tree for so long a time and the vitality of the bud having been preserved under such apparently adverse conditions.

It is so rare for an accurate record of the history of a cut branch of a tree which has been used as a prop to be kept, that the occurrence described above is probably unique and thus is of great botanical interest.

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ON A METHOD OF STAINING THE VASCULAR BUNDLES IN THE LIVING PLANT.—The usual method of tracing the vascular connexions in a stem, viz. by serial sections, is in the main both difficult and laborious. It requires special care in the orientation of the sections, and definite precision in recognizing the particular strands with which the observer is working.

While some experiments on transpiration were being carried out the following simple method of staining the bundles of the living plant was devised. The method serves a double purpose, and enables one (1) to trace the connexions of the vascular strands, and (2) to demonstrate to students the position of the bundles in a growing plant having a simple type of leaf arrangement such as. the decussate.
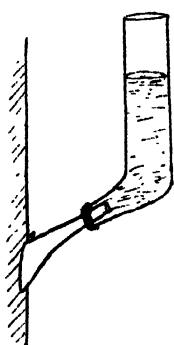
The procedure is as follows : A petiole of a growing pot plant is cut about half-way upward from its base and the cut end is inserted into the end of a glass tube of the shape shown in Fig. 1, and of about 10–20 c.c. capacity. The tube is filled with a dilute solution of a suitable stain, and the plant is then placed under conditions to stimulate transpiration. After a short time (from 1 to 4 hours) it is found that all the strands connected with those of the petiole are stained.

FIG. 1.

The following interesting facts will be noted :

(1) That the xylem is the tissue stained, though a certain amount of stain will have diffused through to the other tissues if the plant has been left too long.

(2) That only common bundles have been stained—the cauline bundles are unchanged.

(3) That below the insertion of the petiole the xylem of the bundles is stained as deeply as above.[1]

(4) That the living cells at the cut end of the petiole may be plasmolysed, if the stain used has been in a strong solution, but that in spite of the exosmosis from these cells the stain has entered the xylem and has been carried from the cut ends right to the apex of the plant.

The most suitable stain, in that it does not affect the protoplasm of the living cells, is a dilute aqueous solution of methylene blue. A dilute aqueous solution of eosin ($\frac{1}{4}$ per cent.) is also a convenient stain, but tends to kill the parenchymatous cells around the bundles. Many other stains may be used if the vascular bundles alone are to be stained, but they may affect the protoplasm of the surrounding cells. Of

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[1] Compare Dixon : The Transpiration Stream, chap. iii, pp. 59–60, 1924.

course this is no disadvantage, since the bundles are stained before the stain passes to the surrounding tissues and destroys their protoplasm. Again, as noted above, only those bundles are stained which are definitely connected with the vascular bundles of the petiole selected. Methylene blue was the stain most used in these experiments, for the reason stated. It gives a dark blue colour to the xylem elements,
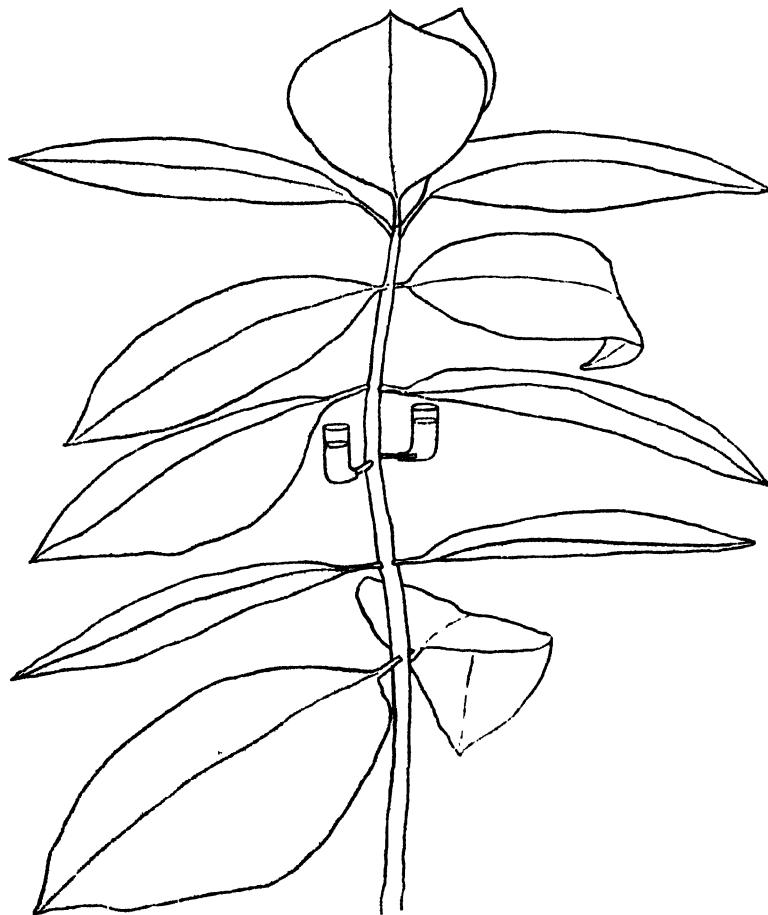
FIG. 2.

and, owing to its rapid action, does not pass into the living cells unless the experiment is carried on over too long a period.

A simple and effective method of demonstrating to students the arrangement of the bundles is by using a plant having decussate phyllotaxis and large thin laminae, e.g. *Hydrangea*. The petioles of two opposite leaves are selected, a tube being attached to the cut end of each. Into one tube is put a solution of eosin, into the other a solution of methylene blue. After a few hours under suitable conditions all

the laminae vertically above and below the eosin tube are distinctly red, as are the corresponding half-laminae of the alternate leaves. The leaves on the other side and the other half-laminae are coloured blue. The plant makes an ideal demonstration of what has been aptly termed a ' physiological chimaera ' (Fig. 2).

Sectioning reveals the fact that the cauline bundles are unstained.

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NOTE ON THE OCCURRENCE OF CLAMP CONNEXIONS IN HIR-NEOLA AURICULA-JUDAE.—Brefeld[1] germinated basidiospores of *Auricularia sambucina* in dilute culture solutions and obtained a luxuriant mycelium on which he did not find any clamp connexions.
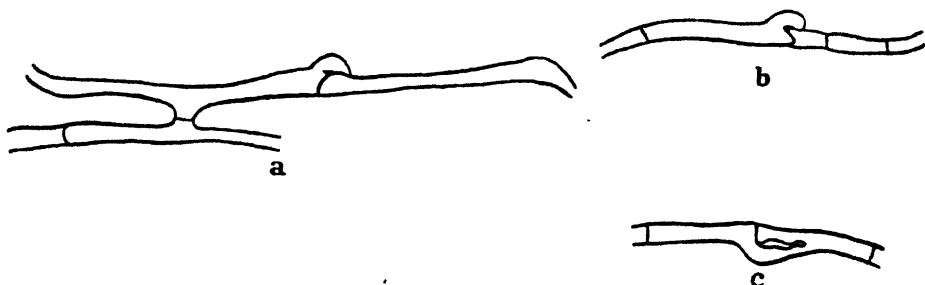
a, b, c. Clamp connexions in *Hirneola auricula-judae.* × 660.

Sappin-Trouffy,[2] in the section devoted to *Auricularia auricula-judae* in his ' Recherches mycologiques ', neither mentions the occurrence of clamp connexions in this fungus nor shows them in his figures.

During the past year, specimens of *Hirneola auricula-judae* were fixed in Flemming's strong solution diluted with an equal volume of water. Some of the material was dehydrated and embedded in paraffin ; the remainder was kept in Calberla's fluid and used for ' squash ' preparations.

The best results were obtained by staining both the microtome sections and the ' squash ' preparations with Heidenhain's iron-haematoxylin, using erythrosin in clove oil as a counter-stain.

In all the preparations numerous clamp connexions were found (see figure).

It is well known that basidia occur only on one surface of the fructifications of *Hirneola.* The clamp connexions are especially numerous towards the sterile surface, and occur in diminishing numbers towards the hymenial layer, the appearance of

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[1] Fortsetzung der Schimmel- und Hefenpilze, vii. 74.
[2] Recherches mycologiques. Le Botaniste, v. 53.

a clamp between a basidium and the sub-terminal cell being very rarely observed. No branches from the clamps have been found.

The cells of the vegetative hyphae frequently contain several deeply staining granules, and for this reason the nuclei are difficult to recognize with certainty, and their relationship to the clamp has not been ascertained. It is evident, however, that many of the clamps are empty, and some are in open continuity with both the adjoining cells.

In his paper, mentioned above, Sappin-Trouffy states that the fungus is composed of hyphae with binucleate cells, the nuclei usually being reduced to specks of chromatin ('de simples taches chromatiques '). Putting aside the question as to whether such structures are nuclei, this observation has not been confirmed in the preparations examined during the present study. Some cells appear to have only one deeply staining granule, others two, while the majority have several and many are empty.

ETHEL GREEN.

BIRKBECK COLLEGE,
June 1924.

Cytology of the Ascomycetes. Pustularia bolarioides Ramsb.

I. Spore Development.

BY

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With Plates V-VIII and three Figures in the Text.

CONTENTS.

Q

I. Introduction.[1]

OUR fundamental knowledge of the cytology of the Ascomycetes has been rendered almost complete by the researches of Dangeard (20, 21, 22), Harper (52, 53, 54, 55, 56, 57, 58), Maire (62, 63), Guillermond (49, 50, 51), Blackman and Fraser (7, 8), Claussen (15, 16), Fraser (36, 37), Fraser and Brooks (41), and Fraser and Welsford (43). It is now admitted that the Ascomycetes have a sexual process, however modified and reduced, in their life-cycle. It is also admitted that there is a process of reduction of chromosomes which in all essential details follows the scheme of reduction of chromosomes of the meiotic phase of animals and plants.

The Ascomycetes in general exhibit an alternation of generations which can be compared in all important details with that of the higher plants. The two generations are not very sharply separated from one another, and the separation line may be shifted in the life-cycle of the members of different families.

Researches on the life-history of the Green Algae (2), Brown Algae (32, 86), Red Algae (85), Rusts (6, 9, 14), Mosses (35, 64), and Ferns (30) have thrown sufficient light on the question of alternation of generations in general. A comparative study of the life-history of these organisms shows that the cytological alternation of generations is not always restricted to morphologically differentiated structures. In the lower groups of plants one is struck with the great diversity of the organs where reduction takes place, while in the other groups the representative units of the two generations may be included in an organ which is morphologically and physiologically identical. The Ascomycetes, with certain modifications, present a type that falls into the last category.

The doubling of the number of chromosomes takes place during the process of fusion of the sexual nuclei. This fusion is completed in the ascogenous hyphae, and the ascogenous hyphae containing the paired nuclei corresponds to the sporophyte, and the young ascus with the definitive nucleus to the spore mother-cell. The sporophytic generation is terminated by the divisions in the ascus, which precede the formation of ascospores when the numerical reduction of chromosomes takes place. The spore on germination produces mycelium, which corresponds to the gametophyte, and in the case of highly sexual Ascomycetes the sexual organs, the homologues of antheridia and the archegonia, are borne on it and normal fertilization is effected, while in the absence of these sexual organs or their homologues

[1] In naming this fungus I am indebted to Mr. J. Ramsbottom, M.A., F.L.S., President of the British Mycological Society, for his kindness in supplying me with the names of the nearest generic and specific allies, as well as the authorities on which this identification is based. The fungus will be fully described in a later publication.

there is, naturally, a wide range of modification of the process by which the two sexual units are brought together. The cycle of alternation of generations is thus complete when we reach the ascogenous hyphae with paired nuclei, the sporophyte.

The complete scheme of alternation of generations in the life-history of the Ascomycetes was first advanced by Harper (58); later on it has been supported by Overton (70) and Strasburger (82, 83), and more recently by Claussen (16). There are, however, several important points of controversy in the detailed account of the nuclear history.

Harper (57, 58), Blackman and Fraser (7, 8), Blackman and Welsford (10), Fraser (36, 37, 39), Fraser and Welsford (43), Fraser and Brooks (41), Cutting (17), Carruthers (13), and Claussen (15) hold that there are two nuclear fusions in the life-history of the Ascomycetes. The first of these two fusions has a true sexual significance. It may take place between the normal gametophytic nuclei of the antheridium and ascogonium. Such processes of normal fertilization in the Ascomycetes have been reported by Harper in *Sphaerotheca* (53, 54), *Erysiphe* (54), *Pyronema* (57), and *Phyllactinia* (58), by Barker in *Monascus* (3), and by Blackman and Fraser in *Sphaerotheca* (7). The absence of such functional male organ involves a reduced sexual process. Fusion takes place between the nuclei of the same female organs. This may again take place in the presence of an abortive male organ, as is recorded in *Ascobolus furfuraceus* by Miss Welsford (84), in *Lachnea stercorea* by Fraser (36), in *Aspergillus repens* by Dale (19); or the male organ may be entirely absent, as in the case of *Humaria granulata* recorded by Blackman and Fraser (8), and in *Lachnea cretea* by Fraser (39).

Similar abnormal sexual process, 'where the sexual fusion of gametes is replaced by a fusion of ordinary gametophytic nuclei which morphologically are not sexually differentiated', has been previously described by Farmer and Digby (30), who worked on Ferns, as *Pseudo-aspogamy*. Certain cases of extreme modification of this process have been discovered by Fraser (37) in *Humaria rutilans*, and by Carruthers in *Helvella crispa* (13), where, in the absence of the ascogonium, the gametophytic nuclei are supplied by the vegetative hyphae of the hypothecium. Such phenomena, as exhibited by *Humaria rutilans* and *Helvella crispa*, where a hypothecial nucleus migrates into an adjoining cell to effect an apogamous fusion, have been defined by Fraser and Chambers (42) as *Pseudogamy*; and this has been compared to the reduced sexual process observed by Farmer and Digby (30) in the prothallium of *Lastrea pseudomas*, var. *polydactyla*.

The nature of the subsequent fusion of the nuclei is 'asexual' (Harper (58), Blackman and Fraser (7), and Fraser (37, &c.)), and its significance is to bring about the 'nucleo-cytoplasmic equilibrium' (Harper (58)) in the ascus.

Thus it may be seen that, according to this doctrine, the fusion in the ascus is always preceded by a fusion in the ascogonium or in an organ homologous to it. As a consequence of the two nuclear fusions, it is claimed that the chromosomes are subjected to two reduction processes (Fraser (37, &c.)); the first of which is *meiotic*, when a true numerical reduction takes place as laid down by Farmer and Moore (31) for the reduction of chromosomes during the meiotic phase of animals and plants. The succeeding division is *brachymeiotic*, which, 'as it lacks a second contraction, admits of less variation in its products than meiosis, and implies either the separation of entire nuclei which fused or at any rate sorting of unaltered chromosomes' (Fraser and Welsford (43)).

Dangeard (22, 23) and Maire (63), however, are in agreement on the question of sexual fusion at the origin of the definitive nucleus, but, contrary to the hypothesis of Harper, Blackman, and Fraser, they have maintained that there is only one nuclear fusion in the life-history of the Ascomycetes, which is followed by one reduction of chromosomes. Maire has explained that the association of the nuclei takes place in the ascogonium. The nuclei divide conjugately, as some sexual nuclei of animals. The fusion of the paired nuclei takes place in the ascogenous hyphae. He has designated this process by the name of *synkarion*. He has thus brought the sexuality of the Ascomycetes into line with the Basidiomycetes (62). The later discoveries of Blackman (6, 9) and Christman (14) on the formation of synkarion in the Uredineae have given support to the hypothesis of Maire.

Claussen (16) has reworked the life-history of *Pyronema confluens* in great detail, and has upheld Maire's observations on the synkarion formation of ascus nuclei. The paired nuclei travel along the ascogenous hyphae and ultimately migrate into the crosier of the young ascus, where they divide, and a *single* union between the descendants of the sexual pronuclei takes place in the young ascus. As regards the three divisions of the ascus nuclei, Claussen supports Guilliermond and others who disagree with two reductions, and confirms the view that the number of chromosomes remains the same in the metaphases as well as in the telophases of all the three divisions; according to his contention there is no sufficient basis for the hypothesis of *brachymeiosis*.

Faull (34) has strongly supported Claussen's view of nuclear migration from antheridium to oogonium where pairing of nuclei takes place, and is followed by a series of conjugate divisions and final fusion of the sexual nuclei in the young ascus; while on the ground of his own observations on the cytology of *Laboulbenia chaetophora* and *L. Gyrinidarum* he has called in question the phenomenon of second reduction.

Following Claussen's work, Shikorra has reworked the life-history of *Monascus* (78) and Ramlow that of *Ascobolus furfuraceus* and *Ascophanus*

carneus (72), and both have confirmed Claussen's observation with regard to the single nuclear fusion in the life-history of the Ascomycetes; and Strasburger (88) has criticized the theory of *brachymeiosis* of Fraser, and has given his support in favour of Claussen.

It seemed, therefore, that the problem was open to further investigation, and a study of the cytology of the Ascomycetes with improved knowledge of technique might help to reconcile the two different views. The following research explains to a certain extent some of the discordant views of the two accounts, and at the same time extends our rather limited knowledge of the nuclear phenomenon in the life-history of the Ascomycetes.

Material. The fungus under investigation was first found at Oxshott, Surrey, in October 1922, on a foray held by the British Mycological Society for students of the London Colleges. Since that time it has been found repeatedly at Oxshott, on Farnham Common and Stoke Common, Bucks., and at Mitcham, Surrey. Invariably it has been found associated with *Epilobium angustifolium*, most frequently being found immediately beneath it. It is of a beautiful salmon-pink colour, though occasionally, in a much younger state, and, very rarely, in well-developed cups, it is somewhat paler. Its nearest ally is *Humaria bolaris* Bresadela ('Fungi Tridentini', ii, p. 73 t.; cxciii, f. 1, 1898), which it resembles in colour and in microscopic structure, but differs from in the size of the parts; for example, it has smaller spores. The generic name is used in the sense of Boudier ('Histoire et Classification des Discomycetes de l'Europe').

II. METHODS.

Some of the more common fixatives were first employed, their dilutions as well as the time for which they were used being carefully worked out. Flemming's strong fluid, Flemming's strong fluid diluted with equal parts of water, and Flemming's weak fluid produced more or less the same effect on the chromatin, when the time exposure in case of the weaker fluids was almost doubled. The cytoplasm was not, however, fixed equally well in both the cases. The stronger fluids made the cytoplasm coarser, which was detected even by staining with haematoxylin, while the two weaker solutions gave it a finer appearance.

Hermann's fluid diluted with equal parts of water was found very satisfactory when the time allowed for exposure was extended to from twenty-four to thirty hours. Later on, it was found that the osmic acid in that solution could be reduced to half its normal quantity without affecting the fixation at all, while at the same time the material was less blackened. It is very difficult to clear the thick cytoplasm of the ascus from the osmic acid, and, unless the minimum amount of it is used, it always leaves a greyish colour, even after the use of hydrogen peroxide for a considerable time.

The difference in the behaviour of two lots of material, one fixed in Hermann's fluid diluted to half and the other in Flemming's weak fluid, was then compared. In a precipitation stain like Heidenhain's haematoxylin the difference was not so very appreciable, and as a matter of fact the cytoplasm of the material fixed in weak Flemming's was finer, while the chromatin appeared equally homogeneous ; but when the material was subjected to a transparent stain, like Breinl or the triple combination of Flemming, the difference was clearly apparent. It was found that chromatin appeared very uniform, and the differentiation of chromatin and linin is much better seen in the material fixed in Hermann's fluid. The cytoplasm, on the other hand, took an intermediate position between the coarseness of material fixed with Flemming's strong fluid and the fineness of that fixed with weak Flemming's fluid. A balance was thus achieved between the precipitation of chromatin and linin inside and the cytoplasm outside the nucleus. The material fixed in half-strength Hermann's fluid, with half the normal quantity of osmic acid, was then restricted to the latter part of the work. The material was fixed mostly in the field, with the aid of an air-pump to secure proper infiltration of the fixing fluid ; while a vast quantity of very young material was brought in the evening into the laboratory on large sods. This was kept under a bell-jar in the greenhouse, and was fixed at intervals of an hour during the night in order to ascertain the state of the nuclei at that time.

The older apothecia were taken through different grades of alcohol, cleared in xylol, and embedded in paraffin in the ordinary way recommended for cytological investigations. The young ascocarps were taken through 10 per cent. glycerin and slowly evaporated to pure, as recommended by Blackman (6) and Miss Digby (28). From pure glycerin they were taken through four grades of glycerin-alcohol mixture to absolute alcohol, and were cleared in cedar-wood oil. The whole process of clearing and embedding was completed within four to five hours' time.

Though Heidenhain's haematoxylin-stained preparations, with or without a counter-stain of either orange G or erythrosine in clove oil, were used in drawing many of the figures of the prophase stage, the results were always checked by a Breinl or Flemming's triple-stain preparation. These preparations were used throughout the investigation in counting the number of chromosomes. When Gram's iodine was used as mordant the chromatin took a deeper violet and the linin retained a considerable amount of blue stain of the methylene polychrome, thus giving a good contrast. This was found useful, as the chromosomes are exceedingly small, and this violet shade was convenient for counting them. The safranin used was matured with a few drops of anilin oil ; this method helped the chromatin to retain the safranin longer, as anilin oil was used for the process of clearing the Breinl preparation.

As the greater portion of the nucleus could be included in a 10 μ thick section, the prophase figures when not otherwise stated have been drawn from 10 and 8 μ sections. Figures of first division of the ascus have been drawn from 8 and 6 μ sections, figures of the second division from 6 μ sections, while 5 and 4 μ sections have been used in counting chromosomes of the second and third telophase and for the study of the spore formation.

III. The Heterotype or First Meiotic Division.[1]

(a) *First Contraction.*

Soon after the fusion of the two gametic nuclei, the definitive nucleus of the ascus, which is the result of this union, passes into a contraction phase. The definitive nucleus at this stage presents a character which resembles in all essential details that of phanerogams and higher cryptogams. The chromatin element is embedded in a condensed matrix of linin and is balled into a conglomerate mass (Pl. V, Fig. 1, a). In a favourably stained Breinl preparation. the chromosomes can be differentiated from the linin matrix, though it seems to be impossible to ascertain their number correctly. The nucleolus is of perfectly spherical shape and is very prominent at this stage. It stains deeply, and is attached to one side of the chromatin aggregate and is not in any way hidden inside it. The chromatic ' ball ' moves to a side of the nucleus and is separated from the delicate nuclear membrane on three sides by clear space, while it is attached to it on one side : in other words, the chromatin is polarized. The cytoplasm of the ascus is fine-grained, and is vacuolated towards the upper part of the ascus. A few faintly stained extruded chromatin bodies are seen passing out of this mass and are degenerating in the vacuolated cytoplasm of the ascus.

The stage of first contraction when the nuclear element is massed into a tight knot lasts only for a short time. This, perhaps, explains why it has so often been overlooked by other workers on the cytology of the Ascomycetes. Fraser (37) is of opinion that in *Humaria rutilans* the nuclei undergo first contraction before they unite to form a definitive nucleus ; while in *Lachnea stercorea* (41) the definitive nucleus passes into the first contraction phase after the fusion in the ascus, and this is followed by second contraction.

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[1] In order to describe this phenomenon, it seems advisable to follow the conventional terminology used to explain the meiotic phases of animals and plants in the strict sense ; it will be seen when following the nuclear history that the sequence of events of different stages in *Pustularia* follows closely the corresponding stages of the phenomenon in the higher plants and animals. The terms *thread, filament, association, dissociation, conjunction,* and *disjunction* are used in the same sense in which Miss Digby (28) defined them in her paper on *Osmunda.* The word *reticulum* has been used in a general sense where the spireme loses its definite character and thus cannot be clearly traced.

Occasionally during the early prophase stages cases of bilateral massing of the spireme, as shown by Pl. V, Fig. 2, have been noticed. They resemble Fraser's Fig. 53 for *Humaria rutilans* (37). In such cases the two separate spiremes which come together after the nuclear fusion lie in the nuclear cavity like two coils of thread bridged over by cross-connexions. Nuclei showing this kind of irregularity have been seen only three or four times and have not therefore given sufficient opportunity for further observation. As they have not been seen in any other transition stages, it seems quite possible that they may have lost this peculiarity as soon as the spireme opens out. They show only delayed fusion of the nuclear contents.

(b) *Synapsis and Hollow-spireme Stage.*

As the nucleus comes out of the first contraction stage, there appear open spaces in the linin substratum. The spireme opens out in distinct parallel threads, which are bounded here and there by the vacuolated space thus formed. The chromosomes which are embedded in the parallel linin gradually become separated from one another. Their number at the earliest stage of the opening of the knot can be determined in well-stained Breinl preparations. There are over twenty-eight bean-shaped chromosomes strung in a broad band of linin thread which can be more or less accurately counted in the stage shown by the bigger ascus nucleus of Pl. V, Fig. 1, *b*. From this stage onwards there is a gradual enlargement of the nuclear cavity.

As the spireme opens out farther, the length of the loops increases and the parallelism of the thread becomes increasingly evident. The uniformly bean-shaped chromosomes split up into smaller chromatin beads of unequal size and shape, which are strung serially on the parallel thread. When the loop has attained a considerable length, it undergoes a twist in the middle and the head of the loop bends over the parallel arms on to the twist (Pl. V, Fig. 3). The nucleolus at this stage moves to the side remotest from the main mass of chromatin. As soon as the folding of the first loop is completed, others, which are visible in the initial stage in the main mass of the spireme, begin to open out. The number of chromatin beads increases as the process of formation of these loops advances. The spireme emerges out from the tangle of synapsis, and the lumpy mass is reduced to a condensed aggregation of short loops which retain a polarized appearance for a considerable time (Pl. V, Fig. 4). The nucleolus becomes entangled inside the loops, and it can be seen distinctly from Pl. V, Fig. 4 that the spireme forms a continuous series of loops without any free end.

The synaptic knot continues to unfold and the loops distribute themselves in the nuclear cavity in parallel series (Pl. V, Fig. 5). The chromatin beads are separated farther apart from each other than they were during

the earlier stages of the opening of the spireme. The nucleus attains its maximum size at this stage. The opening of the spireme does not keep pace with the extension of the nuclear cavity, consequently the spireme is not uniformly distributed in the beginning. The nucleus has an oval shape, and the first-formed loop occupies the long axis of the nucleus, while the others distribute themselves on both sides of it. A fully opened spireme after contraction is shown by Pl. V, Fig. 6. At this stage, the spireme is uniformly distributed in the nuclear cavity. The loops retain the parallel arrangement of their distribution in a more or less elongated area of the nucleus. There are about seven loops at this stage. Two of them in Pl. V, Fig. 6 are to be seen in the same focus. They are more prominent than the others, and appear as if they were interlocked. One of them, slightly the bigger of the two, a, has undergone a twist, while the other, b, has only formed an open loop with crossed arms, but has not undergone any twisting. This shows that the process of looping and twisting may not take place simultaneously. The two arms run parallel almost the whole length of the nucleus, and towards the lower side they are covered by the nucleolus and cannot be farther traced.

The chromatin beads are arranged in a linear series in a thick and rather broad band of linin which completely encloses them. The size and shape of the individual beads can be more readily determined at this stage. Their shape varies a good deal. Some are bean-shaped, others appear slightly oblong or rod-shaped, while a third kind approximate to the triangular. These last, when they occur at the bends of the loops, protrude from the linin band and are conical in form. The spacing of the beads along the spireme is much more irregular, and it will be seen later that this irregularity increases in the different stages in the course of synapsis. At this stage they are more regular than at any other. Though they are well differentiated by safranin or by Breinl stain from the band of linin, the overlying threads of the spireme which cross each other in different directions render any accurate estimate of the number of these beads impossible. It is very clearly seen at this stage that there is no nuclear membrane. The spireme merges into the cytoplasm. The cytoplasm is uniformly thick towards the upper part of the nucleus and extends to the apex of the ascus, while it is vacuolated and loosely spongy towards the lower part of the nucleus, and lower down the ascus tube it forms only a limiting layer. The extrusion of the chromatin bodies increases considerably at this stage. These bodies are seen to come out from the spireme in masses of big blobs as well as in the shape of small beads. They appear as if carried on a sling of linin and are slowly ejected into cytoplasm outside the nuclear cavity, where, surrounded by a clear space in the cytoplasm, they disintegrate.

The next series of changes that takes place in the spireme is manifested

by its rearrangement. It has been seen that the spireme forms a series of complete loops with a twist in the middle, in which place they generally bend. There is no split to be seen in the thread, and it will be evident from subsequent events that this parallelism in *Pustularia* corresponds with the parallelism of the early heterotype prophase of the higher plants. The spireme rearranges itself in the nuclear cavity in such a fashion that these parallel threads of the loops, which are at this stage far apart, may be brought into association. These series of loops, which have hitherto occupied a parallel position in the nucleus, arrange themselves in such a fashion that the heads of the loops come against the nuclear membrane, while the parallel arms take more or less radial positions. Pl. V, Fig. 7 shows a nucleus in which this new order of arrangement has just begun ; Pl. V, Fig. 8 shows an advanced state of this arrangement ; Pl. V, Fig. 9 exhibits diagrammatically this peripheral looping with radial arms. The nucleus presents an appearance of the open spireme stage of heterotype prophase of the vascular plants, but it is not quite the same. From the nature of the individual chromatin beads it can be readily perceived to be an earlier stage of that series. The association of the chromatin beads has just begun at the twist, while the beads on the parallel arms show a tendency to approach towards each other.

The nucleolus occupies a geometrical centre of the nucleus. The spireme can be easily recognized as an endless one. During the long duration of the heterotype prophase this is the only stage in which the spireme distributes itself uniformly in the nuclear cavity, arranging itself symmetrically with reference to the central nucleolus. It is clearly seen at this stage that the spireme forms seven continuous loops, one of which has a double head. This particular loop, *a*, which appears to be more prominent than the others, can be frequently identified during the later stage of synapsis.

(c) *Later Hollow-spireme Stage.*

The order of arrangement of the spireme when the symmetry of the nuclear element is maintained lasts only for a short time, which is evident from the relatively small number of nuclei showing this stage ; after this, the spireme shows a tendency to mass on one side of the nucleus. The loops are again attached to the nucleolus by their parallel arms (Pl. V, Fig. 10). The shape of the nucleus is changed at this stage. It begins to elongate. The maximum elongation of the nuclear cavity is shown by Pl. V, Fig. 11. It is evident from this figure that the loops which are attached to the nucleolus are under a longitudinal strain or pull. The sides of the loops are drawn closer under the action of this pull. The heads of the loops under these circumstances appear to be overlapping each other. It is quite possible that such behaviour of the spireme is due to a physical phenomenon which is obviously helping the arms of the loop to associate.

The associated beads at the twist can be well recognized, and the association of the chromatin beads in the arms is more marked than at any previous stage. The attachment of the spireme to the nucleolus at this stage not only assists this, but at the same time appears to allow the spireme to take up reserve chromatin which is stored in the nucleolus. The chromatin beads increase in size and colour intensity when the spireme is connected with the nucleolus, and this again is followed by an increased discharge of extruded chromatin into the cytoplasm.

A careful examination of the individual beads of the arms shows that, towards the lower part, where the arms are attached to the nucleolus, the fission between the two beds occasionally remains open. The united beads, on the other hand, become slightly elongated in the direction of the pull. This strain lasts only for a short time. Pl. V, Fig. 12 shows that the longitudinal fission opens out again as the spireme is relieved of the strain. The position of the beads in the loops as well as in the parallel arms at this stage is shown in the more thinly cut nucleus of Pl. V, Fig. 13. Each chromatin bead, whether in the loops or in the arms, faces its *complementary half* of the same size and shape in the corresponding part of the spireme threads, which are at this stage rather far apart.

The spireme undergoes condensation preparatory to the second contraction. This gradually brings about the closing up of the split and consequently the reassociation of the beads. This stage is shown by Pl. V, Fig. 14 in an uncut nucleus. The chromatin beads, which will play an important part later on in a series of stages in the formation of bivalent chromosomes, become very prominent at this stage. They show a very remarkably paired arrangement. The paired beads, which have been considered as univalent, can be seen in that part of the spireme which has undergone association, and are very easily distinguished at this stage from the unpaired half-univalent ones. For instance, the beads *a* and *a'* at the crossing of the double-headed loop are two whole univalent beads, each of which has resulted from the association of two half-univalent beads. The beads *b* and *b'* are in the process of association. They are to be seen in all intermediate stages of union and are accommodated on a wider band of linin. The average counts of the chromatin beads in an uncut nucleus, such as is shown in Pl. V, Fig. 14, show that there are about ten united and about forty-four half-univalent beads, or there are approximately sixty-four chromatin beads of half-univalent nature.

A more fortunately cut thin section is shown on Pl. V, Fig. 15. It has a remarkable similarity to Fig. 20 of *Humaria rutilans*, which Fraser (37) has described as a case of second contraction. But though the nature of the spireme in both cases has a close resemblance, the sequence of events which these two figures represent is quite different. According to Fraser's description, that figure shows the loops of the second contraction in which

the longitudinal split between the half-univalent threads has become obliterated, each arm of the loop thus representing a full somatic chromosome. According to our contention, it is at this stage that the premeiotic split is closing up, consequently each arm represents half-univalent or somatic thread only. In other words, her figure showing the pairing of the univalent during the second contraction corresponds, in our seriation of *Pustularia*, with the heterotype prophase when the early parallelism of the spireme is closing up.

The fission and association of the threads give to the spireme the appearance of a longitudinal split. It is evident from our figure that the optical plane of the arms is at right angles to that of the loops ; only the parallel three pairs of arms are shown on the lower side of the nucleus. The heads of the loops have been carried away in another section. Each pair of arms is terminated by a blob of chromatin, which represents the whole univalent bead, the junction of the loop and the arm. Towards the upper part of the nucleus there is one complete loop and parts of other three. Pl. V, Fig. 16 shows almost the same stage, displaying more of the marginal loops than of the radial arms. Pl. V, Fig. 17 represents a thin medium section of the nucleus at this stage.

(d) *Second Contraction.*

During the next stage the hollow spireme draws itself together more closely. As this process advances the union of the parallel arms becomes more intimate, but the fission in the loops still remains open (Pl. V, Fig. 18). The nuclear cavity decreases considerably. At a later stage (Pl. V, Fig. 19), when the closing up of the fission in the loops advances a step farther, the chromatin and linin elements undergo the maximum amount of condensation. It is difficult to trace the spireme at this stage. The loops retain their connexion with the nucleolus.

The fission in the loops is almost obliterated and the association of the univalent halves of the beads has been almost completely secured at this stage. This is indicated by the greater number of big univalent beads, though at the bends of the loops two parallel beads may now and then be observed still remaining apart. In the lower part of the nucleus some beads stained less intensely may be seen carried by a loose matrix of linin. It is quite possible that these beads are afterwards ejected out of the nuclear cavity.

The fission opens out again (Pl. V, Fig. 20), at first gradually, keeping the uniformity of the spireme intact, but as this process advances (Pl. V, Fig. 21) this uniformity is soon lost ; the beads become less and less chromatic. In the same part of the thread a very darkly stained pair of beads is observed, followed by another pair of less strongly stained beads, apparently without any order of arrangement. In other words, the chromatin

becomes very 'rugged' in appearance. That this is a very characteristic stage leading to second contraction has been confirmed by many students of the cytology of higher plants. Lewis (61) found a rugged appearance of spireme in *Pinus* and *Thuja* just before the second contraction, while others have described this phenomenon as a display of conspicuous longitudinal fission by the spireme during the hollow-spireme stage.

A similar process, prior to second contraction, has been described in most of the Ascomycetes whose cytology has been fully investigated. Maire (63) in *Galactinia succosa, Morchella esculenta,* Guilliermond (49, 51) in *Humaria rutilans* and *Peziza vesiculosa,* Fraser (37) in *Humaria rutilans.* Fraser and Welsford (43), Fraser and Brooks (41), have all observed the phenomenon of the spireme undergoing longitudinal fission before entering on second contraction.

The stage presenting fission in the spireme before second contraction has been shown by Fraser (37) in *Humaria rutilans* (Fig. 19) and by Guilliermond (51) in the same fungus (Fig. 56). The post-synaptic fission in *Humaria* has been shown by Fraser in Fig. 22, while an identical stage has been described by Guilliermond (Fig. 57) as synapsis or second contraction. Guilliermond's Fig. 57 is similar to our Pl. VI, Fig. 27 of second contraction in this respect, that while the chromatin is massed on one side of the nuclear cavity, the bare linin below shows no fission. It seems as if here there is a want of harmony in sequence between these two accounts of the second contraction of the same fungus. We have already remarked that Fraser's second contraction stage corresponds to the hollow-spireme stage of *Pustularia.* It is evident, since it shows very marked longitudinal fission and polarization of chromatin, that her Fig. 22 really represents an earlier stage than that shown by our Pls. V and VI, Figs. 24 to 26. It is quite possible that, after missing the first contraction phase, which she supposes the paired nuclei to have passed through independently before their fusion, the subsequent events which she has followed so critically have been, consequently, pushed forward a stage beyond their true sequences. Such a misinterpretation would easily occur, since in *Humaria,* unlike *Pustularia,* there are no regular beads of chromatin to guide the true seriation of successive events. In *Humaria* the chromatin is granular and, as such, it is more or less uniformly distributed on the linin-matrix, while in *Pustularia* definite bodies are passed on from stage to stage, and a change in their arrangement and nature signalizes a definite change in the general history of the nucleus.

The chromatin loses its stainable capacity rapidly, and the spireme is soon reduced to a skeleton after this stage (Pl. V, Fig. 22). As the dechromatization of the spireme advances, the details of the nuclear constituents are almost lost. Nothing of the old spireme can be recognized except a confused maze of linin, speckled with faintly stainable beads. The reticulum appears almost hyaline, and great difficulty is experienced in tracing it, even

with well-differentiated preparations. Towards the periphery of the nucleus the reticulum displays a split here and there which can be detected under a careful focus, though it seems impossible to trace the split any distance. It cannot, therefore, be determined whether the spireme displays the split throughout or not. The distribution of the reticulum is not uniform ; it is more thickly distributed towards the upper part of the nucleolus. Towards the lower part of the nucleus, below the nucleolus, where the reticulum is sparsely distributed, one can easily detect free ends. Pl. V, Fig. 22 shows a favourable nucleus of this stage, indicating that the destruction of the old spireme is almost complete, and that at the same time the reconstruction preparatory for the bivalent state has just begun. The evidence in support of the above statement can be found in a careful analysis of the constituents of this nucleus. In the upper left quadrant of the nucleus there is a small portion of the old spireme which can be identified by its former colour intensity. In the lower left quadrant there is a group of four faintly stained beads in the form of a close ring ; and the right upper quadrant shows the same kind of ring, but formed of more deeply stained beads. Here, then, is the evidence in support of the view that a new arrangement of chromatin beads is taking place in the spireme preparatory to second contraction. In following the reconstruction stages, it will be seen that this grouping of chromatin beads on a linin framework in the form of a ring increases, and each group provides the building material for the construction of bivalents. As a matter of fact, here is the most convincing evidence for the theoretical starting-point of the formation of the bivalents with a fresh grouping of chromatin.

As the nucleus evolves out of this network towards the reconstruction stage, the outline of the underlying structure once again becomes visible. A fine uncut nucleus of this transition period is shown by Pl. V, Fig. 23. The linin is split longitudinally, though the fission is not continuous, being united at different points of the reticulum. The points of union are often marked by a pair of beads attached to one another, and grouping with the pair next to them. The line of original fission between the half-univalents opens out partially from one side and the two beads keep their attachment at the point of association, while by the opened-out ends they are conjoined to the similar pair of half-univalents facing them (Text-fig. 1). The space thus formed by the fission between the two pairs of half-univalents together with the conjunction of the two univalents is enclosed within the ring. The early indication of grouping to form a close ring has been shown in Pl. V, Fig. 22. In Pl. V, Fig. 23 there are three such complete groups, and two others in the initial stage. The distribution of these chromatin groups shows a certain amount of polarity.

The tetrad-like grouping of chromatin proceeds, and we get an arrangement as in Pl. V, Fig. 24. The individual chromatin beads of the tetrads
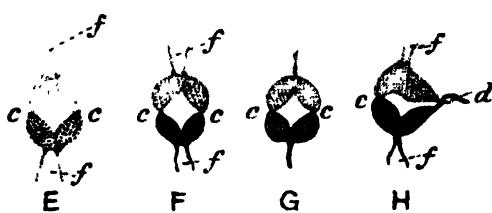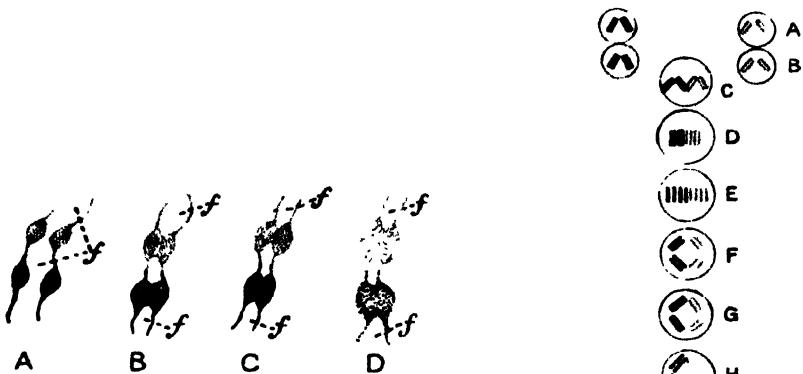
TEXT-FIG. 1.
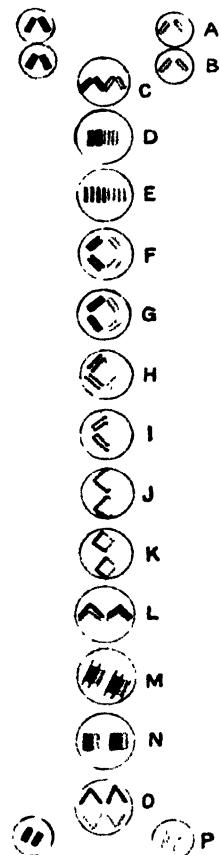
TEXT-FIG. 1. Diagrammatic representation of the chromatin beads showing the formation of the tetrad chromosomes.

A. Early heterotype prophase. B. Early hollow-spireme stage. C. Later hollow-spireme stage. D and E. Disorganization of the spireme prior to second contraction. F. Early stage of second contraction; fission still visible in linin framework. G. Second contraction; disappearance of fission from linin framework. H. Bivalent, showing distortion of tetrad figure, reappearance of fission in the linin framework. f, fission; c, conjunction; d, disjunction.

For the convenience of representation, red and black have been used for the two chromosomes forming the bivalent combination.

TEXT-FIG. 2. Diagrammatic representation of the distribution of chromatin from the ascogonium to the telophase of the first division.

A. Ascogonium. B. Ascogenous hyphae. C. Fusion in the young ascus. D (Pl. V, Fig. 1). Definitive nucleus showing first contraction. E Figs. 3 to 6. Opening out of first contraction with wide fission. F (Figs. 7 to 9). Early hollow spireme; rearrangement of spireme; sorting of half-univalents. G (Figs. 10 to 19). Later hollow spireme: association of half-univalents. H (Figs. 20 to 22). Reappearance of longitudinal fission; disorganization of spireme prior to second contraction. I and J (Figs. 23 to 25). Conjunctions of univalents. K (Figs. 26 to 27). Second contraction; conjunction and fission; appearance of tetrad figures. L (Fig. 28). Early diakinesis; folding of bivalents. M (Figs. 29 to 37). Late diakinesis: twisting and condensation of chromosomes; fission in linin endings visible. N (Fig. 38). Heterotype prophase; equatorial plate stage. O (Figs. 39 to 42). Metaphase and anaphase; appearance of split in the univalents receding towards the pole. P (Figs. 43 to 46). Late anaphase and telophase; disappearance of the split.

For the convenience of representation, red and black have been used for the two sets of chromosomes, each set consisting of two chromosomes.

enlarge, and consequently the two beads of the pair unite, or they may occasionally undergo a slight elongation in one direction. This kind of variation leads to a certain amount of distortion of the ring. Thus it may be noticed that pairs of elongated and blob-like masses of chromatin are facing each other; they tend to form a distinct zone on the linin framework. This, to a certain extent, is due to the condensation of chromatin, and also to the tendency of the individual chromatin beads to group together. As a result of this arrangement the linin framework is laid bare on one side of the nucleus, where it shows the longitudinal split very clearly.

The next stage in advance is shown by Pl. VI, Fig. 25; here the chromatin groups are closer to each other, the blob-like character being accentuated. The nucleolus shows the earliest sign of vacuolization. The zonation of the groups of chromatin becomes more pronounced as the second contraction reaches its maximum (Pl. VI, Fig. 26). In this stage the fusion and condensation of the chromatin beads have increased to such an extent that the space in the ring has become almost obliterated. The half-univalent beads, as they coalesce with each other, enlarge and become extremely blob-like, and cannot therefore be recognized at this stage. Owing to close massing in a comparatively small area, the groups overlie each other. It becomes difficult to identify them at the central region of the nucleus, but when focused on the periphery or towards the linin support they are easily recognizable. The chromatin masses, each stained as deeply as its neighbour, stand out of the reticulum in such a way as to present an impression that these bodies are independent of one another.

Just before the groups separate from one another, the parallel linin unites, and the fission, carried on so far, then disappears entirely. The linin appears as uniformly thick spokes (Pl. VI, Fig. 27) holding out the cluster of tetrads, which are again tied to one another by similar connexions of linin thread. The almost diagrammatic regularity in the form of the tetrads is very noticeable in this drawing, and has not been at all exaggerated. The nucleolus shows a group of vacuoles coalesced together, forming a system of short-branched vacuoles.

(c) Chromosome Formation and Diakinesis.

As the nucleus reaches the highest stage of development of the second contraction, the limit and orientation of the chromosome bivalents can be more readily determined. The apparently quadrivalent arrangement of the chromatin beads at this stage shows the striking regularity with which the pairs are arranged, in an end-to-end fashion, to form a bivalent (Pl. VI, Fig. 27). Though the fission is obliterated at this stage and cannot be seen until the chromosomes begin to separate, the disjunction or the line of separation of the bivalent into two entire univalents is very clearly seen in most of the groups.

In some of the groups it seems that the enclosed space in the tetrads has become almost obscured through the swelling of the beads, and the bivalents appear as a homogeneous mass. But when such groups are carefully focused, the centre of these apparently homogeneous bodies always reveals a more lightly stained area. The chromosome at the upper extremity of the nucleus (Pl. VI, Fig. 27) and that lying below the nucleolus show these characteristics. In other groups, where the disjunction is widely displayed, the arrangement is slightly different. Here it appears as if the two univalents are conjoined at one end only. The other two ends of the pair run parallel for a short distance, and undergo twisting by their linin endings. This phenomenon is shown by two chromosomes of the right inferior quadrant ; cf. Pl. VI, Fig. 27.

It has been observed in the heterotype prophase of the Angiosperms, as well as in that of the vascular Cryptogams, that some of the chromosomes at least appear in definite form when the spireme emerges from second contraction. The general history of the chromosome evolution in *Pustularia* in no way differs from that of higher plants. A characteristic nucleus of this stage is shown by Pl. VI, Fig. 28. The fission in the spireme opens out again as the chromatin concentrates, but from the nature of the fission it is rather difficult to distinguish the post-synaptic from the presynaptic stage, which in these fungi rapidly succeed each other, while the highest stage of development of the second contraction phase intercalated between the two is of much shorter duration. Moreover, the nuclei in these fungi are so small and give such a small amount of chromatin to guide one in working out the true sequence of events that, unless other important characteristics are taken into account, there is a risk of misinterpretation of these two stages which have entirely different significance in the history of chromosome evolution. The chromatin spireme which is coming out of second contraction no longer appears as a simple condensation of homogeneous chromatin granules or an aggregation of beads, as is generally the case prior to second contraction, but always presents a heterogeneity of form. The bivalents, some of them at all events, are seen to be sorting out of the second contraction.

A detailed study of a nucleus at this stage (Pl. VI, Fig. 28) will support the above statement. The figure shows that some of the chromosomes are present in definite forms at the end of the second contraction stage. Two chromosomes, occupying the left superior and inferior quadrants, have just moved away from the main body of the spireme; they have an elongated appearance and are twisted in the middle. There are three others, two lying below the nucleolus and the third above it, which have retained the tetrad arrangement. The rest are clearly in the transition stage, from the ring-shaped grouping of the tetrad to the rather elongated form of the chromosomes. During this stage of progressive differentiation of the chro-

mosomes the nuclear cavity undergoes extension, and this helps the chromosomes to move apart from one another. The linin framework which holds the chromosomes together displays considerable thinness here and there.

The process of segmentation of the chromosomes is accompanied by the phenomenon of torsion (Pl. VI, Fig. 29). The double spireme, in which the limits of the chromosome bivalents have already been laid down, shows a remarkable tendency to undergo folding and twisting. The strain of twisting is transmitted to the linin connexions, so much so, that the connecting string becomes twisted as well as elongated, and is forced to give way in regions which become visibly thin.

As the spireme segments, the chromosomes, which are attached to one another by a loose connexion of linin, show a tendency to move towards the periphery. The chromosome (Pl. VI, Fig. 29) in the left upper quadrant has almost severed its connexion with the main spireme. The character of the chromatin shows clearly as four masses of chromatin placed on the double linin, which is again twisted in the middle. The chromosomes in the lower half of the nucleus show this phenomenon of twisting in all degrees. The same figure also shows that some of the chromosomes retain their regular tetrad form remarkably well, without undergoing any distortion. They can be recognized in almost every nucleus in which chromosomes are forming out of the second contraction spireme till later diakinesis. These chromosomes evidently pass off unchanged, and are to be seen as tetrads during diakinesis.

As the chromatin concentrates, the underlying framework of linin, which quite clearly is split, can be more readily studied. The chromosomes at this stage display very definitely four linin endings. The chromosome occupying the upper left quadrant of Pl. VI, Fig. 29 shows two long and two short ends. But at a later stage (Pl. VI, Fig. 30), when the concentration of chromatin has proceeded a step farther, one has little doubt as to the significance of the four threads of linin coming out of the chromosomes which occupy the upper left quadrant of the nucleus. The fission of the prophase—which emerged between the widely separated parallel arms and loops, was temporarily associated in the later hollow spireme, reopened at the time of derangement of the spireme prior to second contraction, and reassociated during the second contraction—is visible once more at this stage. The split that has been observed in the higher plants in the loop of the post-synaptic spireme, simulating the appearance of true longitudinal fission, can be seen between the thickly twisted arms of the same pair of chromosomes. It is also apparent from the behaviour of this pair of chromosomes that the univalents have united in the plane of the twist.

As the progressive differentiation and condensation of chromosomes advances, the bivalents show a tendency to distribute themselves towards

the periphery (Pl. VI, Fig. 31). It is possible to identify at this stage sixteen pairs of chromosomes. At a later stage the bivalents, as most characteristic of early diakinesis, are distributed uniformly throughout the nuclear cavity. The chromosomes during the early diakinesis present very varied shapes and forms, such as are often to be seen in the Phanerogams and higher Cryptogams, but when mature they appear as bean-shaped or short rod-shaped bodies with moderate variation in size. What appears most striking during this stage is the occurrence of some of them as a close group of chromosome tetrads. These tetrads can be identified through different stages of condensation. The appearance of such tetrads during the early diakinesis is shown on Pl. VI, Fig. 31. Evidently it is one of the constant tetrads which we have observed before, and remains true to its form when the other chromosomes differentiate out from the second contraction. The further modifications of these tetrads are quite simple. Owing to subsequent condensation of linin the chromatin beads approach close to one another (Pl. VI, Fig. 32), and the rectangular form of the pair is occasionally transformed into a close ring. At a still later stage (Pl. VI, Fig. 33) these bivalents appear in the form, so well known to cytologists, of tetrads of the heterotype chromosomes, when four distinct chromatin beads, which are fixed on a square frame of linin and are attached to one another, present themselves in a quadrivalent appearance. Further identification of these tetrads during the later diakinetic stage was not possible ; they might have ended in solid oblong pairs of chromosomes. Of the other forms, V and Y, with widely separated arms and a pair of dumb-bell-shaped chromosomes, are easily recognized through the successive stages of condensation.

The formation of tetrad chromosomes in plants, though not a very common phenomenon, has, nevertheless, been noticed in very widely separated members of different families. They are rather common in the Bryophytes as well as in the Pteridophytes. Moore (66) has figured tetrads in the heterotype division of *Pallavicinia Lyellii*, and Melin (64) in *Sphagnum squarrosum*, and Florin (35) in *Chilocyphus polyanthus*. In the Pteridophytes, Osterhout (69) has observed chromosomes forming regular tetrads or groups of four (' Vierergruppen ') during diakinesis in *Equisetum limosum*. Calkins (12) has described the ' ring type of tetrads ' in *Pteris* and *Adiantum*. Sarbadhikari (76) has noticed similar forms in *Doodia*. In Phanerogams, tetrads appearing as close rings have been described by von Stomps (80) in *Spinacia oleracia* ; and Miss Digby (26) has observed undoubted tetrads in *Primula kewensis*, which appear as quadrivalent arrangements of chromatin.

As has been observed in the case of vascular plants, the two individuals of the bivalent remain widely separated even to a very late stage of diakinesis. The linin connexions between the bivalents become longer and

more delicate, and last to a very late stage of diakinesis (Pl. VI, Figs. 31 to 34). Gradually they become loose, and ultimately become invisible when the centrosomes appear.

(f) *Metaphase, Anaphase, and Telophase.*

* Spindle formation in the Ascomycetes has been studied in great detail by Harper in *Erysiphe* (55) and *Phyllactinia* (58), by Guilliermond in *Peziza rutilans* and in *Pustularia vesiculosa* (49), by Maire in *Galactinia succosa* (63), by Fraser in *Humaria rutilans* (37), and by Claussen in *Pyronema confluens* (16). They all agree as to the intranuclear origin of the centrosome. In *Pustularia* the centrosome is minute and has the shape of a very small, not at all prominent, disc. Unless the preparation is rather overstained the centrosomes cannot easily be detected; very often they can only be traced by following the converging rays of the spindle. It could not, therefore, be determined whether they originated independently or by division of one centrosome, as observed by Maire (63) and Guillermond (51) in *Galactinia succosa*, and by Harper in *Erysiphe* (55) and *Phyllactinia* (58). They appear, however, very near each other (Pl. VII, Fig. 35) and throw out a cone of rays. The chromosomes, which are dispersed uniformly in the nuclear cavity, arrange themselves in the form of a wreath in the region between the centrosome radiations ; and at the same time some of the delicate rays are seen to approach them. The centrosomes later on move apart from each other (Pl. VI, Fig. 36), when connecting rays appear between them. The chromosomes close up and their shape becomes more uniform; the split in the bivalents almost disappears at this stage. The centrosomes move farther apart and a ' Hermann spindle ' is formed between them, and the chromosomes appear to be laterally placed on it (Pl. VI, Fig. 37). At a still later stage (Pl. VI, Fig. 38), when the centrosomes move farthest apart, the chromosomes are drawn into the middle of the spindle to form an equatorial plate. The chromosomes in the equatorial plate stage present the shapes of fat beans or oblong blocks, and slightly elongated rods ; they stain uniformly and show no split.

The metaphase is a stage of long duration, and therefore one can get a sufficient number of nuclei in every slide to follow the separation of the univalents. In Pl. VI, Fig. 39 about five bivalents can be recognized, and the rest are univalents moving towards the poles. At a later stage, when the chromosomes advance towards the poles, a second longitudinal split can be detected in them. The chromosomes split up lengthwise and remain attached to one another by a short or slightly drawn out connexion, and occasionally present a V-shaped appearance (Pl. VI, Figs. 39 to 41). During the anaphase stage (Pl. VII, Fig. 42), as the chromosomes approach nearer to the poles, the halves of the univalent unite ; and at the late anaphase (Pl. VI, Fig. 43), when the chromosomes migrate to the poles in two

batches, they appear again as regular bean-shaped bodies, and the split is seldom visible. The chromosomes do not arrive at the poles all in a body ; at the telophase (Pl. VI, Figs. 44 and 45) one notices some of them lagging behind, and some of these slowly moving chromosomes are to be seen near the middle of the spindle and in all other intermediate positions (Pl. VI, Figs. 44 and 45). No split is visible in them. At the same time, those that arrive at the poles in advance become aggregated ; they swell up and occasionally become attached to one another to form a compact mass. Thus, once they arrive at the poles, during the telophase, their apparent individuality is lost.

On Pl. VI, Fig. 44, which has been drawn from a very well stained Breinl preparation, thirteen chromatic masses can be counted at one pole and fourteen at the other. Of these thirteen bodies one is evidently twice as large as the others ; while at a still later stage (Pl. VI, Fig. 45) any accurate counting of these chromatic masses seems impossible, and an attempt to suggest the nature of their union would be equally unwise. An oblique polar view of a late telophase is shown on Pl. VI, Fig. 46. It shows clearly that the identity of some of these chromosomes can, to a certain extent, be traced to those lumps, but most of them are in different stages of disorganization. The two arms of the chromatic masses are therefore not two arms of a V-shaped chromosome, nor is the opening between them the split in the univalent. One of these lumps, which is the largest of all, obviously represents a swollen chromosome on the point of disintegration. The achromatic spindle forms a thick sheaf of fibres connecting the conglomerate mass at the two poles. The nucleolus becomes elongated and discharges chromatin bodies, which are all scattered in the neighbourhood. It does not stain uniformly and has a thick central core which thins out towards the margin (Pl. VI, Fig. 47). Vacuoles appear in the cytoplasm, which gradually spread out and help the connecting fibres to dissolve (Pl. VI, Fig. 48).

IV. The Second and Third Divisions.

(a) *The Reconstruction of Daughter Nuclei.*

Simultaneously with the thinning out of the achromatic fibres of the spindle there appear two vacuolated regions facing the chromatic masses (Pl. VII, Fig. 48). At this stage, a set of fine linin threads emerging from the periphery of the chromatic masses gradually encloses these vacuolated areas (Pl. VI, Fig. 48 and Pl. VII, Fig. 62). A delicate nuclear membrane is thus formed. The linin thread opens out and forms a fine network in the nuclear cavity (Pl. VI, Fig. 49 and Pl. VII, Fig. 63). At the same time the chromatic mass

breaks up into fine granules, which are eventually distributed in the interstices of the reticulum (Pl. VII, Figs. 50 and 64). At a later stage, when this process advances, the chromatin becomes more or less uniformly distributed in the reticulum (Pl. VII, Figs. 51 and 64). The size of the chromatin masses varies at first, owing to conditions of growth, but when they are fully developed, they show a certain amount of variation in shape and size (Pl. VII, Figs. 51 and 65). The youngest nucleus lies generally uppermost in the ascus, while the intermediates, in the third prophase, range between them. This is the most frequent order of development during the prophase stages; but later on, when spindle-formation takes place, this order is often seen to be reversed.

(b) *The Prophase of the Second and Third Divisions.*

The fine reticulum of the early prophases of the second and third division is gradually transformed into a more or less uniform but coarse spireme (Pl. VII, Figs. 51 and 64). The chromatin beads, which are connected by linin thread, become prominent at this stage. At a later stage (Pl. VII, Fig. 51, lower nucleus) these beads show a tendency to pair. This process of pairing of the beads as a preparation for a contraction phase is very clearly shown by the upper nucleus of Pl. VII, Fig. 52. The prophase of the second division is quickly over, while that of the third division is a long-lasting one; consequently, one can readily observe in the four nuclei of the third division a complete series showing the beads in different degrees of union prior to contraction. Otherwise the process in both the prophases is the same and is subject to the same interpretation.

The development from a uniformly coarse spireme with prominent chromatin beads to a stage of approaching contraction, with the inter-mediate stages, can be seen in a single ascus (Pl. VII, Fig. 65). The number of the chromatin beads which can be more or less accurately determined at this stage is over thirty. They are strung on a broad band of linin and gradually approach one another to undergo association. At a later stage (Pl. VII, Figs. 52 and 66) the parallel threads of linin carrying the half-univalent beads of chromatin come closer together, and this process gradually leads to a remarkable contraction phenomenon. The two nuclei of Pl. VII, Fig. 52 and the four nuclei of Pl. VII, Fig. 66 show the process of association of these beads. The uppermost nucleus of Fig. 66 is in an earlier stage than the lower three nuclei. The three lower ones of Fig. 66 and the one of Pl. VII, Fig. 52 are at the height of contraction. The lower two nuclei of Pl. VII, Fig. 66 show contraction, a fact which clearly demonstrates the nature of the spireme at this stage; it forms a knot with an open end.

The spireme breaks up and the chromosomes, which are sixteen in number (Pl. VII, Fig. 53), are seen to arrange themselves in a wreath-like

fashion in the nuclear cavity. The centrosomes are visible at this stage. To begin with, they appear close to each other, and then throw out a cone of radiations like the first meiotic division. Later on they move apart and the interconnecting fibres appear ; they then move farther apart and a regular spindle is formed. The chromosomes are drawn to the equatorial plate of the metaphase (Pl. VII, Fig. 54). An exactly similar phenomenon takes place when the spireme divides into sixteen chromosomes after the contraction of the third prophase, only in this case it passes slowly, and one can thus follow more easily the details of the process. The chromosomes are distributed uniformly in the nuclear cavity (Pl. VII, Fig. 67), imitating the diakinetic stage of the first division (Pl. VI, Fig. 34).

The chromosomes at this stage present an appearance similar to that of the diakinetic chromosomes of the first division, and have wide splits between their halves. The significance of this split is, however, entirely different in the two cases. In the first division, the split represents the line of *transverse division* of two *entire* chromosomes, which have evolved through a distinct phase of second contraction when tetrads were formed by telosynaptic conjunction of the whole univalents together with the fission between the half-univalents. The split is closed up later on, and the chromosomes become longitudinally united. The significance of the apparently diakinetic figure of the third division, which is followed by a single contraction phase, is easy to interpret, for, like the premeiotic contraction phase, it merely brings about the association of the half-univalents. The split, consequently, represents the line of *longitudinal fission* of the *half-univalent beads* of chromatin which are to function during the succeeding metaphases. The split between the half-univalents closes up gradually and the stages of union can be seen in Pl. VII, Fig. 67. During the union of the two halves they form V-shaped chromosomes with different angles of divergence between the halves. There exists a certain amount of linin fibre loosely attached to these chromosomes at this stage. Later on, very faint centrosomic corpuscles appear (Pl. VIII, Fig. 68) and the chromosomes are drawn on the achromatic spindle like the first and second divisions. The splits in the chromosomes entirely disappear and the chromosomes present thick bean-shaped forms.

Guilliermond (49) has observed the formation of the chromatic knot in the prophase of second division in *Peziza (Humaria) rutilans*, when the chromosomes are united to form a mass in the centre of the nucleus out of which sixteen V-shaped chromosomes arise. Maire (63) has noticed the formation of a chromatic knot in the prophases of the second and third divisions in *Galactinia succosa* and *Peziza vesiculosa*. A stage of contraction prior to the third division has been noticed by Fraser and Welsford (48) in *Otidia aurantia*. In *Peziza vesiculosa* the same authors have again observed this phenomenon in both the second and third divisions. Fraser

and Brooks (41) have described a similar phenomencn of contraction in the prophase of the second and third divisions in *Ascobolus furfuraceus*, which they have compared ' to the so-called first contraction of meiosis '.

(Q) *Metaphase, Anaphase, and Telophase.*

The number of chromosomes at the prophases of the last two divisions is sixteen. They appear uniformly as bean-shaped bodies. The spindle is symmetrical, like that of the first division, and consists of fine fibres (Pl. VII, Fig. 54 and Pl. VIII, Fig. 69).

During the metaphase stage of the second division, the chromosomes split up along the line of union of the half-univalent beads. The process of division of the chromosomes can be readily followed. Pl. VII, Fig. 55 represents such a stage and gives a longitudinal view of the spindle. The upper nucleus of Pl. VII, Fig. 56 presents a polar view of such a stage: the chromosomes in this nucleus are in all different degrees of fission. To begin with, as the chromosomes split up into beads, the beads form an acute **V**; the beads move farther apart from one another, making a wider angle between them. Just before their complete separation they appear as two short rod-shaped or slightly bead-shaped bodies connected by a narrow portion. Pl. VII, Fig. 57 shows the late metaphase stage, when the chromosomes are uniformly distributed all over the spindle.

As the chromosomes move towards the poles, a second longitudinal fission appears in them (Pl. VII, Figs. 55, 58, and 59), which soon closes up as they advance towards the pole. The chromosomes divide into two batches, and each batch, consisting of sixteen chromosomes, moves towards the poles. The passage of the chromosomes towards the poles during the anaphase stage is slow and exhibits a certain amount of irregularity. It is seen that when one batch of chromosomes is nearing the pole some of the chromosomes of the other batch are lagging behind in the neighbourhood of the equatorial plate. This kind of irregularity, as a matter of fact, gives a better opportunity for the study of the character of the chromosomes during the anaphase and telophase stages. From the slowly moving group of chromosomes of Pl. VII, Fig. 58, it is evident that during the anaphase stage the chromosomes again appear to be split up longitudinally, and the two halves are joined by a narrow region presenting a V-shaped appearance with a wide angle between the arms, while at the other end of the spindle, where the chromosomes present a later stage, no split is to be seen in them. The halves have obviously united to form bean-shaped chromosomes again. Even during the late anaphase stage (Pl. VII, Fig. 59) one occasionally notices a pair of beads connected together.

As in the telophase of the first division, the chromosomes during the telophase of the second as well as of the third divisions become very closely aggregated together as soon as they arrive at the poles, and at the same

time a process of disintegration sets in. Consequently their identity is soon lost. Pl. VII, Fig. 60 shows four groups of chromosomes on two spindles in different stages from anaphase to telophase. In the upper nucleus the spindle is curved; the ends, therefore, present a polar view. A telophase stage on a similarly curved spindle is shown on Pl. VII, Fig. 61. The end presenting a polar aspect shows that the chromosomes are very closely massed together. They are in different degrees of disintegration and the number of these bodies appears, approximately, to be twelve. The reconstruction of the daughter nuclei after the second telophase takes place in the same way as after the first division.

The third division merely repeats the second division in every detail. The separation of the halves during the metaphase takes place on a plan similar to that of the second division. The metaphase is shown in a longitudinal view on Pl. VIII, Fig. 70. The chromosomes separate longitudinally along the line of fission of the half-univalents, which closed up during the telophase of the second division, and opened again during the prophase of the third division. The polar view of a similar stage is shown on Pl. VIII, Fig. 71, which is merely a repetition of Pl. VII, Fig. 56 of the second division. Pl. VIII, Fig. 72 represents a stage of metaphase which is comparable to Pl. VII, Fig. 57 of the second division. The separation of the halves is complete, while a subsequent fission is visible in the chromosomes which are approaching the poles. This fission is closed up quickly during the late anaphase (Pl. VIII, Fig. 73), when one can count two batches, each of sixteen bean-shaped chromosomes, moving towards the poles. The process of movement of the chromosomes towards the poles, as well as the process of disorganization of the chromosomes at the poles, is much more rapid than in the second division. But as it is often accompanied by a certain amount of irregularity on the part of the chromosomes moving towards the poles, one therefore can find sufficient cases to prove that there is no reduction in the number after the third division. The uppermost nucleus of Pl. VIII, Fig. 74 shows chromosomes at the poles appearing as regular bean-shaped bodies, and the number is about fourteen. The nucleus next below is at a little later stage, and the rest are in the telophase and late telophase stages. Pl. VIII, Fig. 75 shows the polar view of a nucleus at a late telophase stage which can be compared with Pl. VI, Fig. 46 of the first and Pl. VII, Fig. 61 of the second divisions.

V. SPORE FORMATION.

The daughter nuclei are formed in a manner similar to that observed after the first and second divisions. The connecting fibres of the spindle gradually dissolve away and vacuolated spaces appear facing the chromatic lump (Pl. VIII, Fig. 76). At this stage very faint astral radiations are

visible emerging from the periphery of the chromatic lump ; the centro-some is not visible at this stage. It seems to be hidden in the chromatin mass and the emanation of astral rays signifies its presence there. At a later stage the chromatin mass gradually fragments into a number of small chromatic granules which are connected to each other by a delicate linin network (Pl. VIII, Fig. 77). The astral rays become more numerous and prominent. The centrosomes are clearly seen at this stage ; they are apparently much bigger than before, and are disc-shaped. The number of rays increases, and a delicate membrane is formed round the nucleus (Pl. VIII, Fig. 78). The cytoplasm gathers round the nucleus in a rather dense mass, while its distribution becomes considerably thinner in the intervening region. The nuclear membrane is gradually pulled out into a characteristic nuclear beak. At a later stage (Pl. VIII, Fig. 79), when the spores are com-pletely delimited from the hyaline cytoplasm, one can more readily study the mechanism of the underlying process of spore formation. The centro-some throws out a set of rays which are recurved to form an outer and inner series. The outer series opens out as umbrella-like radiations and encloses the cytoplasm as sporoplasm. The inner series of rays are fine and fibrous ; they form the nuclear membrane. The delimitation of the spores is helped by the appearance of vacuoles bordering the spore wall. The shape of the spore is uniformly round at this stage. The nuclear beak is very prominent and the centrosome appears as a small condensed disc and is situated at the apex of the beak.

The method of spore formation in the Ascomycetes has been a subject of divided opinion among the workers on the cytology of the Ascomycetes. Harper (52), who first studied the spore formation in this group of fungi, is of opinion that the spores are delimited by astral rays. He has concluded that the spore wall is formed by the lateral fusion of the astral rays, while the recurved ends of the fibres fuse again in a similar fashion to form the nuclear membrane. In his later work (58) on *Phyllactinia corylea*, as well as on *Erysiphe cichoracearum*, he has confirmed his previous observations on the spore formation. The whole spore body is formed out of undifferentiated cytoplasm of the ascus by the formation of a plasma membrane derived by the lateral fusion of the fibres without the deposition of a cellulose wall. In a recent paper (59) he has again expressed his opinion that the centrosomes in the Ascomycetes originate in the region of the cell where the chromatin and the cytoplasm come into specific contact and ' where fibrillar kinoplasm is formed and passes out to form the plasma membrane of the young daughter-cell, the ascospore '.

Faull (33, 34) denies Harper's conclusion that the ascospore wall originates from the lateral fusion of the astral rays. He concludes that the spores are delimited by the differentiation of a limiting layer of hyaline or finely granular protoplasm. This differentiation begins adjacent to the

centrosome and continues progressively to the other side of the pole. The delimitation of the spore, according to Faull, takes place at the expense of kinoplasm or altered cytoplasm. Faull, in his account, attempts to establish a connexion between the sporangia of the Phycomycetes and the ascus.

Fraser (37, 41, 43) has suggested that though the spores are delimited by astral rays, the lines of rays represent the flow of an enzyme, the centro-some being the centre of these enzymic activities. In *Humaria rutilans* (37), for example, the spore is delimited by astral rays, as Harper (52, 58) suggested, but the nature of these rays suggests the flow of currents set up in the neighbourhood of the centrosome; this she has confirmed later on by her study on the spore formation in *Peziza vesiculosa* (43). In *Ascobolus furfuraceus* Fraser and Brooks (41) have noticed vacuolated areas or line of cleavage delimiting the spore, a phenomenon essentially comparable with that observed by Faull (33) in *Neotiella*. *Lachnea stercorea*, in which the astral rays are well marked, again approaches the forms studied by Harper.

The spores in *Pustularia* are delimited by the astral rays emanating from the centrosome, as Harper (52, 58, 59) stated. The incurved rays which form the nuclear membrane are fine and fibrous, and are to be seen for a very short time. How far they are transformed into nuclear membrane could not therefore be determined. Vacuole formation undoubtedly plays an important part in delimitation of spores. The sporoplasm is as finely granular as the original cytoplasm enclosed by the astral rays, except that it forms a dense layer round the nucleus. No difference could therefore be made out between the sporoplasm and the limiting cytoplasm. Again, from the character of the cytoplasm and from the changing shape of the spore as shown by Pl. VIII, Figs. 78 and 81, it seems quite possible, as Fraser and Brooks (41) have remarked, that a new tension is set up in the neigh-bourhood of the centrosomes which in a great measure accounts for the formation of a cleavage line, and ultimately for the vacuole formation in the cytoplasm delimiting the spore.

The nuclear beak becomes further elongated, and with it the spore elongates (Pl. VIII, Fig. 80). At a later stage (Pl. VIII, Fig. 81) the number of chromatin granules in the reticulum increases, when one can readily count more than twenty-four of them. The beak disappears, and the nucleus takes a rounded shape; the chromatin beads increase in size and number, and become more chromatic (Pl. VIII, Fig. 82). Their number at this stage is over thirty; they are short rod-shaped bodies (Pl. VIII, Fig. 83), as has been seen in the prophase of the second and third divisions, just before contraction. At a later stage (Pl. VIII, Fig. 84) these beads are again connected by a spireme. At the same time the beads are seen to approach one another to form pairs. At first the paired beads form a wide, and then an acute, angle between them, as the union of these beads becomes closer. The split between the two beads forming a **V** closes

up gradually (Pl. VIII, Fig. 85), and the bean-shaped chromosomes become apparent once more. The chromatin is deposited on the linin connexion, and the nuclear cavity gradually begins to decrease. At a later stage (Pl. VIII, Fig. 86) the spireme character becomes more prominent, and the chromosomes appear as swollen nodes on the spireme. The number of the chromosomes at this stage is about sixteen. The thick-noded spireme (Pl. VIII, Fig. 87) gradually contracts away from the nuclear membrane. The sporoplasm thins out on both sides of the nucleus at this stage and the oil glands appear, first on one side of the nucleus and then on the other. The oil cavities increase in size, and the nucleus, which lies in the middle of the spore, becomes elongated in the direction of the shortest diameter of the spore. The sporoplasm becomes a thin limiting layer on both sides of the nucleus facing the oil drops. The spireme, now almost deformed, loses its staining power, and in the mature spore (Pl. VIII, Fig. 89) the resting nucleus often moves to an eccentric position facing the spore wall. The number of the chromosomes of a resting nucleus cannot be determined. The size of the spore varies from $10 \times 14\,\mu$ to $14 \times 20\,\mu$.

VI. DISCUSSION.

(a) *General Considerations.*

In *Pustularia* we face once more some of the most controversial problems of modern cytology, the most outstanding of which is the significance of parallel threads arising from the first contraction knot, as well as the nature of the chromatin beads which are strung on these parallel threads. In *Lilium*, as well as in a large number of other plants, these parallel threads present an appearance of longitudinal fission in the spireme. This longitudinal fission has received at the hands of various investigators very different interpretations, which can be divided into two main groups.

One school of cytologists, notably Farmer and Moore (31), Mottier (67), Schaffner (77), Miss Digby (25, 26, 27, 28), and other botanists, interpret this split as a precocious division of the spireme, which only reaches its consummation during the homotype division of the chromosomes. According to their view, this split is not to be confused with the subsequent split formed by the looping over of the entire spireme during the second contraction. The most potent evidence on which some of the investigators of this school have based their interpretation, is the establishment of the identity of the parallel threads of the last premeiotic division of the sporogenous tissue with the parallel threads of the heterotype prophase. According to these investigators, this premeiotic fission, though occasionally masked by temporary association, is carried through the prophase of the heterotype,

and the final separation leads to the quantitative division of chromatin at the homotype metaphase.

Following the first contraction, the nucleus enters upon a second contraction phenomenon. As the spireme passes into this phase, the longitudinal fission becomes almost obscured, and this is followed by a confusion of the chromatin spireme when the primary fission appears again. As, however, the spireme evolves out of the second contraction, it is thrown out into a number of loops, between the arms of which a second fission appears resembling the true or primary longitudinal fission; but the original longitudinal fission can be occasionally seen in the sides of the loops. Through further condensation the arms of the loops become closely approximated. The loops separate from one another, and the two limbs tend to become twisted about each other. Each arm of the loop consequently corresponds to a single chromosome, and by their approximation the arms form a bivalent chromosome. According to Farmer and Moore, ' the side of the loops represents not the longitudinal halves of a split thread, but the approximation of serially distinct regions of the spireme as a whole '.

The other school of investigators, chiefly supported by Grégoire (47, 48), Berghs (4, 5), Strasburger (82, 83), Allen (1, 2), Miyaki (65), Rosenberg (73 to 75), Overton (71), and others, regard this fission as the longitudinal approximation and conjugation of independent threads of paternal and maternal origin. Berghs (4), in his investigation of the formation of heterotype chromosome in *Allium fistulosum*, has observed that this dual nature of the filament is due to a longitudinal approximation of two filaments, which lead to the formation of a thick spireme. In his earlier works, Strasburger (81) remarked that the explanation of the parallelism of the spireme is to be looked for in the telophase of the last premeiotic division. This interpretation, as a matter of fact, supported the view held by the first school; but later on (82) he changed his opinion. He has subsequently (83) interpreted the double nature of the spireme during heterotype mitosis as a parallel conjugation, and not as an early splitting. He has further stated that the explanation of the reduction from the bivalent to the univalent number is to be found in this parallel conjugation. It follows as a corollary to the above theory that the investigators belonging to the later school do not admit the importance of second contraction in the formation of bivalent chromosomes.

It has already been stated in the introduction that immediately after the union of the gametic nuclei in Ascomycetes, the definitive nucleus which results from this union enters upon the first contraction stage of the heterotype prophase, like the spore mother-cells of higher plants. Consequently here is no independent sporophytic generation in the life-history of such fungi. On the other hand, as the sporophytic nucleus enters abruptly into

the prophase of the heterotype, the series of events, such as premeiotic divisions and complete resting interphases which are noticed in the life-history of most of the Cormophytes, is cut short in the history of the sporo-phytic nucleus. Thus to follow the parallel threads from a preceding division in the sporogenous tissue is impossible. What we are concerned with in *Pustularia* is the later behaviour of the parallel spireme after first contraction, and our interpretation in this matter is to be based upon the subsequent sequence of events in the history of the parallel spireme.

In order to determine what evidence *Pustularia* presents for the signi-ficance of the duality of the spireme in question, it seems advisable to give here a concise account of the behaviour of the chromatin beads from synapsis to the subsequent division stages.

The first contraction knot (Pl. V, Fig. 1) carries the full number of somatic and univalent chromosomes. The knot opens out (Pl. V, Fig. 1) into parallel threads carrying these chromosomes along with them ; and during the earliest stage of the opening of the knot the somatic number can be counted. The threads, as they grow, undergo twisting in the middle, thus forming loops with parallel arms (Pl. V, Figs. 3 to 6). As the process of the opening of the loop advances, the chromosomes split up longitudin-ally, forming chromatin beads. From the fully opened spireme after the first contraction till the hollow-spireme stage (Pl. V, Figs. 6 to 13), the nuclear activity is manifested by a process of rearrangements of the loops to effect the pairing of the *half*-chromatin beads, which, during the process of opening of the knot, have become split up and have been carried away in the loop far apart. During the early hollow-spireme stage the chromatin beads show a tendency to undergo association, and this phenomenon becomes more accentuated during the later hollow-spireme stages (Pl. V, Figs. 14 to 17).

The association of the threads is gradually completed (Pl. V, Fig. 18) and the fission is obliterated (Pl. V, Fig. 19) ; and this is rapidly followed by the reopening of the split (Pl. V, Figs. 20 and 21). The chromatin beads lose their staining power, and the spireme presents a rugged appearance. The loss of chromatin by the spireme, together with the reappearance of the original longitudinal fission, lead to its complete disorganization before the second contraction (Pl. V, Fig. 22). The reconstruction of the bivalents begins very rapidly from this confusion. The beads become increasingly chromatic (Pl. V, Fig. 23). The most characteristic feature of the nucleus entering on the second contraction is the formation of regular tetrad chromosomes from these beads (Pl. V, Fig. 24 and Pl. VI, Figs. 25 to 27). During the sub-sequent stages of segmentation and condensation, up to the early diakinesis (Pl. VI, Figs. 28 to 33), the tetrads appear in the form of rectangles or rings. The univalents separate during the heterotype metaphase (Pl. VI, Fig. 39). During the early anaphase the split is visible again, though for

a short time, in the chromosomes advancing towards the poles. The daughter nuclei are reconstructed during the late telophases, after which a period of rest follows.

The chromatic lump is entirely dissolved away during the period of rest, except for a few minute granules which are to be seen in the hyaline matrix of linin. The spireme is reconstructed during the interphase (Pls. VI and VII, Figs. 49 to 51). The spireme presents again the parallelism which is so characteristic of the heterotype prophase (Pl. VII, Fig. 52). The split is closed up by a simple process of contraction and the halves are again associated together and present the whole univalent chromosome on the homotype spindle (Pl. VII, Figs. 53 and 54). The separation of these halves is effected during the metaphase of the homotype (Pl. VII, Figs. 55 to 58), and the meiotic phase of the nucleus is completed.

During the prophase of the post-meiotic division of the nucleus, the spireme presents once more the phenomenon of parallelism (Pl. VII, Figs. 64 to 66) identical with that of the heterotype prophase and that of the homotype prophase. The split is closed, and the chromatin beads of the spireme are longitudinally associated in pairs by a remarkable contraction phase. The spireme segments and the number of whole univalent chromosomes becomes apparent again during the diakinetic stage (Pl. VII, Fig. 67 and Pl. VIII, Fig. 68) and on the equatorial plate stage of the third division (Pl. VIII, Fig. 69). The split appears again in the chromosomes during the early anaphase (Pl. VIII, Figs. 70 to 72), and during the late telophase the full number of half-univalent beads can again be counted in the spore nuclei (Pl. VIII, Figs. 81 to 84).

The occurrence of the post-meiotic contraction phase, which takes place in the same cell and soon after the heterotype reduction has been completed, is a unique phenomenon in the Ascomycetes which is hardly known in any other organism, and an equivalent for this should be found, if anywhere, in the premeiotic and early heterotype prophase of higher plants. The process involved in these post-synaptic contractions is a simple one, and consequently calls for a simple interpretation. On the other hand, if the explanation of the heterotype reduction, as suggested by Grégoire, Berghs, Strasburger, Allen, Miyaki, Overton, and others, is to be found in the phenomenon of early parallelism of the heterotype prophase (when by the lateral conjugation of parallel threads an apparent numerical reduction of chromosomes to half their number is brought about), there is no substantial reason why this process should be repeated in the history of the daughter nucleus, after its aim has already been achieved.

The above description of the nature of the spireme and the behaviour of the chromatin beads in the spireme during the heterotype prophase, as well as during the succeeding interkinetic phases, presents us with an opportunity of discussing the significance of prochromosomes, and the part they

play in the formation of bivalents. A considerable mass of information has accumulated on this subject. The literature of the discussion has often been quoted in most of the works on cytology where the spireme is described as presenting this aspect. But as, in the case of *Pustularia*, the interpretation of the sequences has been entirely based on the nature and behaviour of these chromatin beads, we are consequently led to a very different conclusion from that put forward, as to the significance of the pro-chromosomes. Thus it seems well worth our while to bring forward some of the most noted views on the subject.

Strasburger (82), in connexion with the formation of heterotype chromosomes in *Lilium*, held the view that the pairing of the homologous maternal and paternal chromosomes takes place in the form of gamosomes. The gamosomes which are present in the somatic cells of *Galtonia* correspond to the somatic number of chromosomes. Miyaki (65), in his investigation of the pollen-formation of certain Monocotyledons, has found concentrated chromatin masses in the presynaptic stage which are comparable to the gamosomes of Strasburger. The gamosomes pair to form zygosomes during the heterotype prophase. Overton (71) has observed prochromosomes in the somatic nuclei as well as in the resting germ-cells of *Thalictrum purpureus* and *Calycanthus floridus*. They are arranged in parallel pairs. He concluded that double spiremes, which are associated during fertilization, remain side by side, and actual conjugation occurs during synapsis or the associated stages. Rosenberg (73) has observed pro-chromosomes in the form of chromatic masses in *Hieracium* and *Tanacetum* during the early heterotype prophase of the pollen mother-cells which are present in somatic numbers. They unite in parallel pairs during synapsis to form gamosome pairs, each member of which is a univalent chromosome. In *Crepis virens* (74) he has noticed a paired arrangement of prochromosomes in the premeiotic resting nucleus, when they are present in diploid number, while in the resting tetrads they are present in haploid number. In the resting somatic cells of *Nuphar* and *Helianthus* (75) he has again observed prochromosomes in the form of chromatic masses which are apparent in somatic number.

Maire (63) has described the formation of granular chromatin bodies in *Galactinia succosa* and *Peziza vesiculosa*, which he has termed protochromosomes. These bodies generally unite two by two, in the prophases of the three divisions, by forming a knot out of which are formed bigger bodies of half the number of these chromatin granules. The protochromosomes which appear in the prophases of *Galactinia* in the form of granules are rather variable and transitory formations. As a matter of fact, Guilliermond (51), in a later research on *Galactinia*, refuses to accept Maire's observation of the union of these bodies to form chromosomes.

Fresh light has been thrown by Miss Digby (27) on the question of the

presence of definite chromatin bodies as prochromosomes in the resting pre-meiotic as well as in the resting tetrad nucleus of *Crepis virens*. She has explained that in *Crepis* there may be stages of complete disintegration of chromatin in the resting nucleus, leading to a distribution of chromatin in the reticulum in very fine form, or the disintegration may be so slight as to allow of the identification of the chromatic aggregations as prochromosomes. As to the numerical relation of these aggregations with the chromosomes, she has found that the numbers of these bodies are inconsistent and variable. Consequently the rigid hypothesis of the permanency of these bodies from one cell generation to another, as put forward by Rosenberg, does not hold good. The chromosomes lose their identity during the interkinetal rest. The reconstruction of the chromatin beads during the early prophase of the second and third divisions indicates a phase of reawakening of the resting nucleus to an active stage. The number of these chromatin beads during the early prophase of the heterotype approximately corresponds to twice the somatic number. Consequently, in view of present research, each bead is equivalent to a half somatic or univalent chromosome. The apparent numerical relation will be further supported by the second part of the discussion. The distribution of the chromatin beads from the first contraction of the early heterotype prophase to the telophase of the first division has been diagrammatically shown by Text-fig. 2.

The occurrence of these bodies in the reticulum of the resting somatic cell or in the prophase of the heterotype division, whether they are in the forms of elongated threads, definite beads, condensed chromatic aggregation, cloudy masses, or otherwise, raises the question of the subsequent arrangement of these bodies in the spireme of the early heterotype prophase and the part they play later on in the formation of the bivalent combination. Miss Digby (25) has made this point clear in her paper on *Galtonia*, and Farmer (29) has emphasized the fact that the main difference of opinion between the two schools lies in the question of the pairing of the univalents, while the rest are merely secondary parts of this main problem.

Having stated our opinion in relation to the significance of the parallelism of the heterotype, we are now able to discuss the importance of second contraction, which, according to the first school of cytologists, is one of the most important corollaries of the main proposition. Farmer and Moore (31) consider this phase as 'essentially a synaptic one', and hold that it is thus primarily involved in the pairing of the homologous portions of the univalent spireme to form the bivalents. This view has been upheld by Lewis (61), Miss Digby (25, &c.), Nothnagel (68), and others. In *Galtonia* (25) and *Osmunda* (28) Miss Digby has observed that the pairing of the entire univalent chromosomes takes place during the second contraction.

In *Pustularia* the second contraction has a marked significance in the

formation of the bivalents. The most striking feature which characterizes the setting in of the second contraction is the gradual sorting out of the chromatin beads into the form of tetrads. Prior to the appearance of the tetrads, the spireme is distinguished by the reopening of the longitudinal fission. This process, together with the telosynaptic conjunction of the univalents, gives rise to tetrad figures. The mode of tetrad formation during the second contraction is a straightforward process. We have been able to show that some of the later modifications of these tetrads during the evolution of heterotype chromosomes are as simple as their origin.

In clearing up the most obscure and difficult problem in the significance of the parallelism of the heterotype prophase in *Osmunda* (28)—as to which interpretations differed so widely—Miss Digby lays emphasis on the point that the separation between two *half-univalent threads* and *conjunction* between two *whole univalents* are two different phenomena which should not be confused. In *Osmunda* the spireme, before entering on the second contraction, shows the phenomenon of conjunction or union of whole univalents very remarkably, but fission in the conjoining filaments is completely obscured. Sarbadhikari (76) has observed in *Doodia* that the vestiges of fission remain apparent throughout the second contraction, and can be traced in the chromosomes of advanced diakinesis, when the fission can be seen in the conjoining univalents of each bivalent combination. *Pustularia*, in this respect, presents an intermediate aspect. Though the split cannot be traced in the linin framework during the second contraction, yet in the chromatin spireme the simultaneous occurrence of partial fission in the half-univalent beads and conjunction between two homologous univalents leads to the formation of tetrads. Calkins (12) has made a similar observation in his investigation of chromatin reduction and tetrad formation in Pteridophytes. According to his description the tetrad is derived by a longitudinal fission of the spireme segments, together with the transverse union of two chromosomes.

Farmer and Moore (31) considered the process of the separation of these tetrads as easier to interpret than their formation. They have explained that these tetrad chromosomes separate as pairs of dyads in the heterotype, whilst in the homotype mitosis each dyad further divides into monads, which are thus distributed between the daughter nuclei of the second division; while Grégoire and others regard these forms as due to quadripartite rather than quadrivalent arrangement of chromatids. According to the latter, a slight constriction in the conjugated univalents may lead to the quadripartite appearance, and as such it has nothing to do with the reduction phenomenon.

It is not difficult to realize how the formation of tetrads in plants during the heterotype prophase strikes at the very root of the arguments of Grégoire and others in favour of the early parasynaptic conjugation of the spireme. Those who maintain that the longitudinal fission of the early

heterotype prophase in plants does not completely close up, but ends in the separation of the univalents on the heterotype spindle, will have to modify their hypothesis with regard to the appearance of a simultaneous split in the spireme at right angles to the conjugating plane that is functioning in the homotype mitosis. Otherwise there will be an insurmountable difficulty in reconciling their hypothesis with the fact of the appearance of tetrad chromosomes in the heterotype prophase. In *Pustularia* the split in question has been traced from the early prophase, and its identity has been established with the parallelism of the early prophase. On this ground, again, their theory to establish the early pairing of the chromosomes by the criterion of parallelism of the heterotype prophase breaks down.

It is interesting to record at this stage an intermediate view held by a number of prominent cytologists, Gates (45, 46), Lawson (60), and Fraser (38) amongst them, who have in recent years worked on the pollen formations. Gates (44) has observed in *Oenothera rubinervis* that chromosomes which are stout, short, and sausage-shaped, come out of the first contraction in pairs, and are arranged in an end-to-end fashion. The spireme segments into pairs and the members of each pair come to lie side by side to form bivalents. In *Lactuca* (46), again, there is no indication of pairing of threads in the early prophase, but the delicate univalent spireme of the first contraction gradually condenses to form the short and thick spireme of the second contraction. The chromosomes are arranged end to end, as in *Oenothera*, and the method of reduction is in accordance with the scheme of Farmer and Moore. Gates is of opinion that looping over can only take place when the chromosomes are long and thread-like, as in *Lactuca* and *Galtonia*. In such cases a phase of second contraction is intercalated to bring about their approximation. In *Oenothera*, on the other hand, there is no need for a phase of second contraction. Though the early heterotype spireme in *Pustularia* presents a remarkable similarity to that of *Oenothera*, the subsequent phases leading to the formation of bivalents differ greatly. The chromosomes, though very small, short, and bean-shaped, nevertheless pass through a very distinct stage of second contraction when the pairing of the homologous paternal and maternal chromosomes is brought about. Fraser (38), though essentially in agreement with Farmer and his supporters on the significance of the parallelism of the early prophase, has found evidence of both parasynaptic and telosynaptic pairing of the thread in the later stages of second contraction in the pollen mother-cells of *Vicia Faba*.

There appears to exist a more general agreement as to the nature and significance of synapsis, as well as the method and formation of bivalents, from the evidence furnished by the Ascomycetes hitherto cytologically investigated. Guilliermond (49) regards the first division in the ascus as heterotype. He has worked out the cytology of *Peziza vesiculosa, Humaria*

rutilans, Galactinia succosa, and *Peziza catinus* in great detail. The spireme often showed a paired arrangement of filaments. The synaptic stages are very distinctly characterized. The chromatin spireme condenses and forms a knot on one side of the nucleus. The chromosomes are formed out of this synaptic knot by side-by-side union of the threads. The heterotype chromosomes during diakinesis present the forms of **V, U, 8, O,** and other forms seen during the heterotype prophase of higher plants. In the metaphase the chromosomes separate in a more or less longitudinal plane, forming hollow lozenges. On their advance towards the pole during anaphase a second longitudinal split appears in them, and the chromosomes appear **V**-shaped again. In his earlier account (49) Guilliermond regarded this phenomenon of reduction as essentially the same as that of pollen formation studied by Grégoire, Berghs, and Strasburger (82). But in a later work (51) he has completed his investigation on the cytology of *Humaria rutilans* by adding some of the most essential figures of the synaptic stages, and has accepted the interpretation of the phenomenon as advanced by Fraser (37).

Maire (63) is inclined to accept the interpretation of the reduction phenomenon in the Ascomycetes as put forward by Strasburger (81) and Farmer and Moore (31). In *Galactinia succosa* the true longitudinal fission closes up, and the union is followed by a folding of the filament and longitudinal fusion which renders the chromatic filament twice as thick as before. The filaments coil themselves into a compact knot which segments into a number of chromosomes.

Fraser and her colleagues (37, &c.) have been able to establish definitely the presence of both the fissions in the heterotype spireme of the Ascomycetes. In *Humaria rutilans, Otidia aurantia, Peziza vesiculosa,* and other forms which they have so critically studied, they have traced the first or true fission till the second contraction. During the second contraction a process of looping over follows. In the arms of these loops, before and after segmentation, they have detected this primary fission, while the fission between the loops represents the line of approximation to the univalents, which are arranged in the spireme in an end-to-end fashion. Some of the early diakinetic bivalents in *Humaria* (Figs. 24 *b* and *c*) present a remarkable similarity to those of *Pustularia* (Pl. VI, Fig. 30). Though occasionally broken by unavoidable gaps, her sequence in *Humaria* is in a general way in agreement with that of *Pustularia.* The evidence she has obtained from *Humaria* and other forms strongly supports the view held by Farmer and Moore. She has remarked that the main difference in the interpretations of the phenomenon advocated by the two aforesaid schools is due to a difference in the true seriation of events. It would seem that this is also, to a certain extent, due to want of harmony in correlating the observed sequences.

(b) *The Second and Third Divisions.*

Passing on to the succeeding divisions from the telophase of the hetero-type, we are confronted with certain phenomena peculiar to these fungi, owing to the intercalated resting stages, but, nevertheless, the processes are comparatively simple. The chromosomes in most of the vascular plants, after telophase, maintain their individuality to a certain extent, and often remain almost intact throughout the process of interkinesis. They may occasionally undergo partial dispersal of their chromatin substance. When the chromosomes are long or thready and of good size, they under-go vacuolization, and thus form semi-reticulated or irregular alveolized bands. A complete rest is seldom seen in the higher plants. Consequently, fewer steps are required for their reconstruction.

In *Pustularia*, as the nuclei undergo a period of rest during interkinesis, which evidently lasts for a considerable time, the process of reconstruction is not quite so simple. The daughter chromosomes cannot be traced farther at the telophase. The chromosomes which are distributed between the daughter nuclei form a chromatin mass which completely dissolves away during the period of rest. Consequently the succeeding phases do not occur till after a considerable period. During the early prophase, the chromatin becomes concentrated in the interstices of the linin, and forms a fine reticulum. The reticulum is transformed into a coarse spireme and the split of the heterotype becomes again evident in the parallel thread of the spireme. The split is closed up and the beads are again associated through a simple process of contraction.

There seems to exist a certain amount of obscurity as to the signifi-cance of the phenomenon of contraction just referred to which has been observed in some other Ascomycetes. The interpretation put forward by some authors to explain this post-meiotic contraction phase has interfered with the views as to the nature and significance of the second division in these fungi, hitherto accepted without question as homotypic in strict agree-ment with the interpretation of this division in animals and in plants. This phenomenon of contraction, as well as a subsequent one prior to the third division, has been partially dealt with in the first part of the discus-sion in connexion with the general trend of the heterotype prophase. But from the point of view of the cytology of the Ascomycetes, as this phenome-non presents us with a matter of further controversy, it seems desirable to discuss the question from this aspect.

According to the observations of Guilliermond (49, 51) in *Peziza (Humaria) rutilans, Peziza catinus*, and *Pustularia (Peziza) vesiculosa*, the chromosomes which separate out of the chromatic knot present the charac-ter of those of the first division, the only difference being that they do not

form the hollow lozenges of the first division at the metaphase. There is a transverse division of the **V**-shaped chromosomes as in the homotype division of the Phanerogams. Maire (63) regards the second division as essentially homotype, which brings about the separation of the half-chromosome thread formed at the metaphase of the preceding division. According to Claussen (16) the chromosome relation in *Pyronema confluens* in the second and third divisions is the same as in the first. The first division differs from the other two by the presence of synapsis and diakinesis of the heterotype. Similar observation has been recorded by Brooks (11) and Faull (34) from their investigation of other groups of Ascomycetes.

Fraser in her work on the cytology of *Humaria rutilans* (37) upheld Guilliermond's interpretation of the second division. But the subsequent discovery of a contraction phenomenon during the prophase of the second division in *Peziza vesiculosa* (43) and *Ascobolus furfuraceus* (41), and the numerical reduction of chromosomes at the telophase, led her to change considerably the interpretation of this division. She has attached importance to this contraction phase combined with a subsequent one preceding the third division, as connected with some kind of pairing arrangement of the allelomorphs, so that these contraction phases are thus ultimately responsible for bringing about the brachymeiotic reduction between the last two divisions. The second division in these forms, therefore, according to her contention, is not a straightforward homotype. but more or less a reducing one.

Our knowledge as to the significance of the first contraction, of the parallelism of the heterotype as well as that of the second contraction, is fortunately definite in this case. At the same time, our observations of this phenomenon during the succeeding phases and the processes which are connected with them are without any break, so that nothing is left obscure. The split has been traced through the interphases to the subsequent contraction. The number of chromatin beads left over from the early prophases and the second and the third divisions (Pl. VII, Figs. 51 and 65) is the same; while the number of chromosomes in the anaphase of the second division (Pl. VII, Fig. 59) agrees with that of the late prophase of the third division (Pl. VII, Fig. 67 and Pl. VIII, Figs. 68 and 69). With these evidences at our disposal we are unable to accept the contention of Fraser and her colleagues that the contraction before the second division is in any way connected with the pairing of the allelomorphs. The second division is homotypic, and is associated with the longitudinal separation of sister halves of the univalent chromosomes, which are represented by these beads precociously united during the prophase of the heterotype. From the extraordinary way in which these beads undergo premature separation in the early prophase spireme after an interkinetic rest, it is obvious that the attachment between the two halves of the somatic chromosomes is not firm enough to hold them together. In these organisms in which these beads are peculiarly liable to

separate from one another it is not unexpected to find a simple contrivance, such as a process of contraction, intercalated to bring about their association.

The third division has been regarded by a considerable number of investigators as equivalent to a vegetative division, like the second, to effect quantitative division of chromatin, which is to be distributed between the spores. On the other hand, Fraser and her colleagues hold that this division is essentially a reducing one, which they have defined as *brachy-meiosis*. The controversy on the nature of this division arose out of cytological investigation of *Humaria rutilans*, which has been worked out by Guilliermond (49) as well as by Fraser (37). Guilliermond has observed sixteen straight chromosomes at the telophase of the third division, while Fraser has regarded them as sixteen ends of eight **V**-shaped chromosomes. Unfortunately, the chromosomes in *Humaria* after the third division become extremely thready, and consequently none of these authors has been able to follow the mode of separation of the chromosome during the metaphase, nor have they obtained a clear view of the anaphase. The interpretation of Fraser and her colleagues of this division in other forms of Ascomycetes investigated by them differs widely from the views of Guilliermond and Maire, who studied the same fungi.

The brachymeiotic reduction, according to Fraser and her colleagues, is a very variable phenomenon, but its most essential feature seems to be to bring about a numerical reduction, when half the number of chromosomes passes to each daughter nucleus. The variability is not only shown by the shifting period through which this reduction takes place, but also by the mechanism by which the reduction is brought about. For instance, this reduction may be effected only during the third division, without any evident pairing by the allelomorphs (*Humaria rutilans*); or it may be preceded by a pairing of allelomorphs which is effected by a contraction phase (*Otidia aurantia*); or such pairing may take place in the prophases of both the second and third divisions (*Peziza vesiculosa* and *Ascobolus furfuraceus*); or the pairing may take place very early in the life-history, after fertilization, in which case the chromosomes appear in half the number in the division of each of the paired nuclei in the crosier (*Humaria granu-lata*). In the absence of any contraction phase which could be related to such pairing, this has been explained as a case of (partial) association of chromosomes. Lastly, the pairing of the allelomorphs may be not at all apparent at any period of the life-history of the nucleus, but association of chromosomes takes place from one division to another (*Phyllactinia corylea*). In such a case there is no change in the chromosome number throughout the life-history of the fungus. It has been argued that the chromosomes remain tetravalent till the first division and bivalent till the third, the association being so intimate as to leave no visible effect of their treble and double nature on the chromosomes.

Though the contraction phase before the third division is very conspicuous, we are unable to confirm any numerical reduction of the chromosomes at this division. The process of separation of the beads is very clear during the metaphase, and in the anaphase the late prophase number of chromosomes is repeated. Consequently, the contraction phenomenon here also is not associated with any allelomorphic pairing of the chromosomes. We gain further insight into the problem by studying the condition of the young spore nuclei. As a matter of fact, the early spireme condition of the spore nuclei shown on Pl. VIII, Figs. 81 to 84, according to the convention observed in flowering plants, represents a late telophase stage, when the chromatin beads are present again in the prophasic number of the third division, while the number of chromosomes in the mature spore corresponds to the number of chromosomes in the late prophase of the third division. The distribution of the chromatin beads from the telophase of the first division to the succeeding divisions, and in the spores, is diagrammatically shown in Text-fig. 3.

Finally, there only remains to be discussed her last contention, the evidence drawn from *Phyllactinia*, and partly also from *Humaria granulata*, in which there is no change in the chromosome number throughout the life-history, as a case of chromosome association rather than true pairing. In *Phyllactinia* (58) at the time of fertilization, the male and female chromosomes approximate in pairs. As each of these two nuclei carries eight chromatin strands and in the united nuclei eight strands of chromatin could be counted, it is assumed that in the absence of any apparent sign of pairing, the paternal and maternal chromosomes, represented by the chromatin strands, have become associated side by side, as the result of which the number remains the same but the valency has been doubled. The second or asexual fusion takes place in the young ascus when these double chromatin strands again become approximated in pairs. This is followed by the synapsis stage, when the whole chromatin mass begins to contract and becomes more dense. After synapsis the chromosomes again appear as eight strands, and ultimately give rise to eight tetravalent chromosomes on the equatorial plate of the first division.

This Harper-Fraser hypothesis of chromosome association, even though it could be given a theoretical consideration, has its drawbacks. According to the above description of synapsis in *Phyllactinia*, one cannot overlook the importance Harper has attached to the process of synapsis that brings about the pairing of the chromosomes and thus causes the actual reduction of chromosome number. The point is how far it is justifiable to correlate this synaptic contraction with the sexual fusion. In fact, in *Phyllactinia* as well as in *Humaria granulata*, the fertilization is followed by no apparent disturbance of the nuclear substance, but the process of actual reduction and the synaptic stages that bring it about are postponed till a subsequent

asexual fusion has taken place. Following the analogy between the phenomenon of fertilization in animals and plants, in which the actual reduction process is related to the visible synaptic stages and the matura-
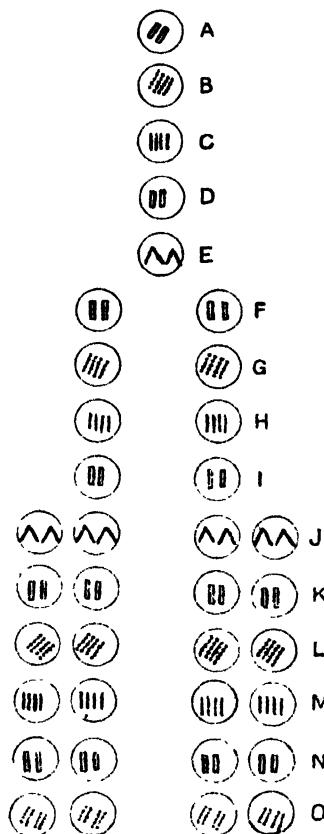
TEXT-FIG. 3. Diagrammatic representation of the distribution of chromatin beads from the telophase of the first division to the succeeding divisions and in the spore. A (Pl. VI, Figs. 43-46). Telophase of the first division. B (Figs. 49-50). Resting stage during interkinesis. C (Figs. 51-52). Prophase of the homotype, showing parallelism of the spireme. D (Figs. 52-54). Late prophases; contraction to equatorial stage; association of half-univalents. E (Figs. 55-58). Metaphase to early anaphase; appearance of split in the chromosomes receding towards the pole. F (Figs. 59-61). Late anaphase and telophase. G (Fig. 63). Interkinetal rest. H (Figs. 64-66). Prophase of the third division, showing parallelism of the spireme. I (Figs. 66-69). Late prophases; contraction to equatorial stage; association of half-univalents. J (Figs. 70-73). Metaphase to anaphase; split visible in some of the chromosomes receding towards the pole. K (Figs. 74, 75). Anaphase to telophase. L and M (Figs. 76-84). Late telophase in the immature spore nucleus. N (Figs. 85, 86). Mature spore nucleus. O (Fig. 89). Resting spore nucleus.

tion divisions succeeding them, and the phenomenon of asexual fusion, in which (as defined by Fraser), without any visible indication of pairing, an intimate association of nuclear substance takes place, which again is followed by a separation of unaltered chromosomes, it appears quite reasonable to

suggest that the two phenomena in question in the life-history of *Phyllactinia* and *Humaria granulata* may have been reversed. If this deduction is correct, then Claussen's hypothesis (16) of association followed by true fusion of the sexual nuclei holds good. In fact, lately Claussen's observation has received confirmation by Shikorra (78) and Ramlow (72), who undertook to rework this part of the problem on other members of the Ascomycetes.

The phenomenon of pairing of the homologous chromosomes and the association of the complementary halves of the univalents are distinctly marked out processes in *Pustularia*. Consequently, the interpretation of *Phyllactinia*, be it as it may, cannot be applied to *Pustularia*.

Claussen (16) has aptly remarked that such a phenomenon as the occurrence of more than two divisions of the spore mother-cells, is not uncommon in plants. Farmer and Williams (32) have stated that reduction of Fucaceae takes place during the first division of the nucleus. The second division follows the first, and after the third or final oogonial mitosis eight eggs are found. Yamanouchi (85) has closely followed the reduction process in *Fucus*. Reduction takes place during the first division of antheridial and oogonial mitosis, after which each of the antheridial nuclei undergoes five more divisions, resulting in the formation of sixty-four sperm mother-cells, while the oogonial nucleus, as Farmer and Williams observed, divides twice and forms eight egg-cells. Similar examples in which spore mother-cells undergo more than two divisions can be quoted from higher plants. In Phanerogams this is seen in the embryo sac of *Lilium*, in which, after heterotype and homotype divisions, there follows rapidly a third division. There is no need to accumulate instances. Besides, some of the spores of the sixteen-spored ascus (Pl. VIII, Fig. 90) appear to possess quite normal nuclei. It is absurd to suggest that in such cases there have been, three times, fusions of the nuclei, which have made the definitive nucleus of the ascus octavalent, and consequently that there have been three reductions to form univalent ascospores.

The above facts and the evidence at our disposal from the study of *Pustularia* forbid us to enter into any such generalization as that the third division is essentially a reducing one. On the other hand, we are of the same opinion as Guilliermond, Claussen, Brooks, Faull, and others, that in all the three divisions the chromosomes are present in the same numerical relation; the first division is heterotype, the second is homotype, and the third is equivalent to a vegetative division. If, however, the question of a second reduction is purely a matter of interpretation, such a difference resolves itself into an unimportant one.

VII. Summary.

1. The definitive nucleus in *Pustularia* presents a very marked contraction phenomenon, wherein the chromatins of the two fused nuclei retain their individuality to a certain extent, as in the case of the first contraction of higher plants.

2. The first contraction knot opens out as a parallel spireme, when the chromosomes split up, and are serially arranged in the linin thread, forming loops. During the subsequent stages of the early heterotype prophase a rearrangement of the spireme is shown by a development which finally brings about the union of the chromatin beads thus split up. The function of the parallel threads of the early heterotype prophase is, therefore, to bring about the association of the chromatin beads which are of a half-univalent nature.

3. Prior to the second contraction, the fission opens out again, and the chromatin spireme undergoes disorganization ; but reconstruction of the spireme takes place speedily, and as the nucleus enters upon the second contraction stage, the fission in the linin frame-work closes up completely, but that in the chromatin beads remains partially open. The process of second contraction is very remarkable in *Pustularia,* and brings about the formation of regular tetrad bivalents owing to fission in half-univalent beads, together with an end-to-end conjunction of the whole univalent.

4. There are sixteen bivalent chromosomes in the prophase of the first division. The first division is heterotype, and the chromosome reduction follows essentially the scheme laid down by Farmer and Moore.

5. The second division is' homotype, and it is succeeded by an interphase and a contraction phenomenon whose function is to bring about the reassociation of the half-univalent beads, which in these fungi are liable to undergo precocious separation.

6. The second division is followed by an interphase, when the nucleus appears to undergo a state of complete rest. The spireme is reconstructed, and the half-univalent beads again appear in full but reduced number.

7. The nuclei present a phenomenon of post-meiotic parallelism, which can be interpreted in the light of the parallelism of early heterotype prophase, which brings about the association of half-univalents. The first contraction phase, as well as those prior to the last two divisions, is not, therefore, associated with any kind of pairing of full somatic chromosomes.

8. The third division is equivalent to a vegetative one, in which the chromosome divides like a somatic division, and the chromatin is distributed in the nuclei of eight spores.

9. The number of chromosomes is the same in the prophases as well as in the telophases of all the three divisions. There is no second reduction in

Pustularia, nor is there any evidence that an association of whole somatic chromosomes has taken place from one nuclear division to another. Owing to the presence of prominent contraction phases during the prophase of every division, such an hypothesis does not apply to *Pustularia.*

10. The number of chromatin beads remains apparently the same in the prophases of the last two divisions, as well as in the young spore nucleus which represents the late telophase stage of the third division. As the spore matures the chromatin beads reunite when the chromosomes appear in the full number of the third prophase. In a resting spore nucleus the chromosomes become disorganized and their individuality is apparently lost.

11. When the spores are formed, the centrosomes play an essential part, throwing out rays which are recurved to form an inner and an outer series. The inner series is transformed into a nuclear membrane, and the outer series encloses cytoplasm.

12. 'Chromatin bodies' are extruded from the nuclear framework. During the heterotype prophase, connexion of the spireme is established with the nucleolus several times, when the extrusion of these bodies reaches its maximum, and occasionally a portion of the spireme is seen to be thrown out into the cytoplasm.

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IX. EXPLANATION OF PLATES V–VIII.

Illustrating Mr. Bagchee's paper on *Pustularia bolarioides* Ramsb.

All the figures were drawn with the aid of an Abbe camera lucida under 2 mm. apochr. hom. imm. Zeiss N.A. 1–40 with comp. oc. 18. × about 2250.

PLATE V.

Heterotype Division.

Fig. 1. Definitive nucleus of ascus: *a*, nucleus, showing first meiotic contraction; massing of the nuclear substance on one side of the nucleus; chromosomes are embedded in the linin matrix; *b*, slightly later stage, showing the opening of the knot; appearance of vacuolated space in the linin matrix.

Fig. 2. Ascus containing single nucleus in which the spireme is concentrated into two masses which are bridged over by cross-connexions.

Fig. 3. Nucleus showing the loosening of the first contraction knot and appearance of the first loop with parallel arms; while others are in the initial stage in the main body of the knot.

Fig. 4. Further stage in loosening of the knot as wide loops, which are formed of beaded chromatin strung in a broad band of linin. The others are concentrated on one side of the nucleus.

Fig. 5. An almost completely opened synaptic knot, showing the endless nature of the spireme, which consists of simple and twisted loops.

Fig. 6. Fully opened spireme after first contraction. Nucleus showing two prominent loops, *a* and *b*, in the same focus; the loop *a* is twisted in the middle, and the loop *b* is a simple loop without any twist.

Fig. 7. Synapsis showing a peripheral arrangement of the loops.

Fig. 8. Early ' hollow-spireme' stage, showing a further stage during the rearrangement of the spireme. The spireme shows the earliest stage of association of the beads at the place of twist in the loops.

Fig. 9. Early 'hollow-spireme' stage, showing the association of the beads has become more pronounced at the twist. *a*, double-headed loop with associated bead at the twist.

Fig. 10. A slightly later stage. Nucleus showing a rearrangement of the spireme. The continuity of the spireme is broken and the loops are attached to the nucleolus. Association of the beads of the arms is apparent.

Fig. 11. Nucleus showing an advanced 'hollow-spireme' stage. Note the strained and elongated appearance of the loops; the united beads have become elongated in the direction of the pull.

Fig. 12. 'Hollow spireme.' The spireme is relieved of the strain. The beads have again undergone fission, but each of them faces its complementary half. Note the migration of chromatin spireme into the cytoplasm.

Fig. 13. Superficial section of a nucleus of the same stage as Fig. 12. Note the parallel arrangement of the complementary beads.

Fig. 14. Later 'hollow-spireme' stage. Nucleus showing accentuated association of the beads. The univalents are very marked at the twist of the double-headed loop, *a* and *a'*, and the two pairs, *b* and *b'*, are in the process of association.

Fig. 15. Superficial section of a nucleus of late ' hollow-spireme ' stage. Nucleus shows more of the parallel arms than of the loops. Fission is closing up. Note *a* and *a'*, the univalent beads at the point of junction of the loop and the arms.

Fig. 16. Another superficial section of a nucleus of the same stage, showing more of the loops than of the parallel arms. Note that the fission of the loops is closing up.

Fig. 17. A thin medium section of a nucleus of the same stage, showing a closer association of the beads.

Fig. 18. Massing of the spireme before the second contraction. The spiremes show closer association of the beads. Note the increase in the number of large-sized beads.

Fig. 19. Slightly later stage, showing that the association of the beads is almost complete. Note that nearly all the beads are large sized.

Fig. 20. Nucleus showing the reopening of the fission before the second contraction. Note the split-up beads at the curve of the loops.

Fig. 21. Nucleus showing a further stage of advance in the process of reopening of the fission. The spireme presents a ' rugged' appearance.

Fig. 22. Nucleus showing a complete disorganization of the spireme prior to second contraction. Note *a*, a portion of the old spireme, and *b* and *b'*, grouping of the chromatin beads as tetrads.

Fig. 23. Commencement of the second contraction stage. The spireme now reconstructed shows fission and pronounced grouping of the beads to form tetrads.

Fig. 24. Nucleus entering upon the second contraction stage. Note the zonation of chromatin on linin. The linin framework shows fission.

PLATE VI.

Fig. 25. Slightly later stage. Nucleus shows an accentuated grouping of the chromatin beads as tetrads.

Fig. 26. Further advance in the second contraction stage. Note the close grouping of the tetrads. Fission in the linin framework is still visible.

Fig. 27. Nucleus in the height of second contraction stage. The fission in the linin framework has completely disappeared. The chromatin tetrads are distinctly polarized. Note the bivalent segments showing fission in the individual beads together with conjunction with the next pair to form a close ring.

Fig. 28. Nucleus showing spireme emerging from the second contraction stage. Note the transformation of the tetrads into intermediate forms of chromosomes.

Fig. 29. Loosening of the second contraction. Note the segmentation of the chromosomes is accompanied by twist and torsion.

Fig. 30. Early diakinesis. Note the original fission visible again between the pairs of linin endings and that the conjoined pairs are twisted in the middle.

Fig. 31. Early diakinesis, showing gradual evolution of the chromosomes after the complete segmentation of the spireme. Note the close ring-form of tetrads, *a*.

Figs. 32 and 33. Later stages of concentration of the chromosomes. Sixteen pairs of chromosomes can be identified. Some of them have already taken up the mature form of bean and the others are in tetrads, *a*, and intermediate forms.

Fig. 34. Diakinesis. Split between the univalent halves of the bivalent combination is still open.

Fig. 35. Diakinesis, showing early stage of spindle formation. Two centrosomes lie close together with a cone of fibres radiating from them.

Fig. 36. Spindle formation. Centrosomes move farther apart from one another. The spindle is established.

Fig. 37. Spindle formation. Appearance of ' Hermann spindle' with laterally placed chromosomes. Note that the split between the univalents has completely disappeared.

Fig. 38. Mature spindle. Chromosomes on the equatorial plate stage. Sixteen mature bivalent chromosomes.

Fig. 39. Early metaphase, showing five bivalents; the rest are univalents. Note the appearance of fission in some of the univalents moving towards the poles.

Fig. 40. Advanced stage of metaphase. Note the fission in some of the univalents.

Fig. 41. Slightly later stage. As the chromosomes move towards the poles in two batches, fission widely separates the halves.

Fig. 42. Early anaphase. Fission, though visible in some of the chromosomes, is rapidly disappearing.

Fig. 43. Anaphase. The fission is no longer visible in the retiring chromosomes.

Fig. 44. Early lophase, showing disorganization of the chromosomes at the poles.

Fig. 45. Telophase. Chromosomes forming a compact mass at the poles.

Fig. 46. Telophase. Oblique polar view of the chromosomes. Notice the different ways these chromosomes disorganize and lose their individuality at the poles during the telophase.

Fig. 47. Telophase. Achromatic fibres forming a bundle-sheaf. Appearance of vacuoles between the chromatic aggregations.

Fig. 48. Later telophase, showing the formation of daughter nuclei.

Second Division.

Fig. 49. Later telophase, showing the reconstruction of the chromatin spireme.

PLATE VII.

Fig. 50. 'Resting' nucleus to fine spireme stage.

Fig. 51. Early prophase. The lower nucleus shows the chromatin beads undergoing association during the coarse spireme stage.

Fig. 52. Advanced prophase. The nuclei show the approach of contraction phase. The association of the beads is clearly shown in the upper nucleus.

Fig. 53. Spindle formation. Centrosomes are near each other in one of the nuclei; the other shows the formation of 'Hermann spindle '.

Fig. 54. Mature spindle. Chromosomes on the equatorial plate, showing polar and longitudinal views.

Fig. 55. Early metaphase. The nucleus shows the separation of chromosomes in longitudinal view. Note the appearance of longitudinal fission in the chromosomes advancing towards the poles.

Fig. 56. The same stage as Fig. 55, showing the separation of chromosomes in polar view. Note the appearance of wide fission during the separation of the chromosomes.

Fig. 57. Metaphase, showing chromosomes distributed all over the spindle.

Fig. 58. Early anaphase. The chromosomes moving towards the poles in two batches. Note the reappearance of wide fission in the chromosomes of the slowly moving batch.

Fig. 59. Anaphase stage, showing fission in the chromosomes has disappeared.

Fig. 60. Anaphase to telophase. Note the disorganization of the chromosomes during the telophase stage.

Fig. 61. Polar view of the telophase, showing chromosomes at different stages of disorganization.

Fig. 62. Late telophase stage, showing the formation of the daughter nuclei.

Third Division.

Fig. 63. 'Resting' nuclei.

Fig. 64. 'Resting' nucleus to fine spireme stage.

Fig. 65. Early prophase stage, showing coarse spireme stage with prominent beads.

Fig. 66. Early prophase stage, showing post-meiotic parallelism of the spireme and contraction phase.

Fig. 67. Prophase, showing the distribution of sixteen chromosomes uniformly in the nuclear cavity. Note that some of the chromosomes present a wide fission and appear as V-shaped bodies.

PLATE VIII.

Fig. 68. Spindle formation. Centrosomes are near one another. Note the bean-shaped form of the mature chromosomes, and that there is no fission in them.

Fig. 69. Prophase, showing chromosomes on the equatorial plate stage in longitudinal, polar, and oblique views.

Fig. 70. Early metaphase stage, showing the separation of the chromosomes in longitudinal view.

Fig. 71. The same stage as Fig. 70, showing the separation of the chromosomes in polar view.

Fig. 72. Metaphase stage, showing the distribution of the chromosomes all over the spindle. Note that some of the chromosomes approaching towards the poles present a wide fission and appear as two beads connected together by a narrow portion.

Fig. 73. Anaphase, showing the chromosomes are separated into two batches. No fission is visible in them.

Fig. 74. Ascus, showing nuclei from anaphase to telophase. Note the disorganization of the chromosomes during the telophase.

Fig. 75. An oblique view of the telophase.

Spore Formation.

Fig. 76. Telophase stage, showing the emanation of astral rays from the periphery of the chromatin mass.

Fig. 77. Late telophase stage, showing the fragmentation of the chromatin mass into beads. Centrosome is separated from the chromatin mass.

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Fig. 78. Early stage of spore delimitation. Formation of nuclear beak. Note the accumulation of cytoplasm round nuclei.

Fig. 79. Spore delimitation, later stage. Note the umbrella-like radiation, the elongated nuclear beak, and the fibrous nature of the nuclear membrane.

Fig. 80. Very young spore. Nuclei showing fine chromatin spireme.

Fig. 81. Slightly later stage of the young spore. Nucleus showing a coarse chromatin spireme. Spore-wall shows distinctly fibrous nature.

Fig. 82. Young spore. Nucleus showing chromatin beads.

Fig. 83. Young spore, another stage.

Fig. 84. Young spore, showing association of the beads and gradual formation of the chromatin spireme in the spore nucleus.

Fig. 85. Later stage of young spore. Nucleus showing the association of the beads is complete.

Fig. 86. Immature spore. Nucleus showing a thick spireme with noded chromatin.

Fig. 87. Immature spore in fine section, showing the formation of oil cavities. Nucleus with deformed spireme.

Fig. 88. Mature spore with fully developed oil cavities. Nucleus showing a skeleton spireme.

Fig. 89. Mature spore with ' resting ' nucleus.

Fig. 90. Spores from an abnormal sixteen-spored ascus.

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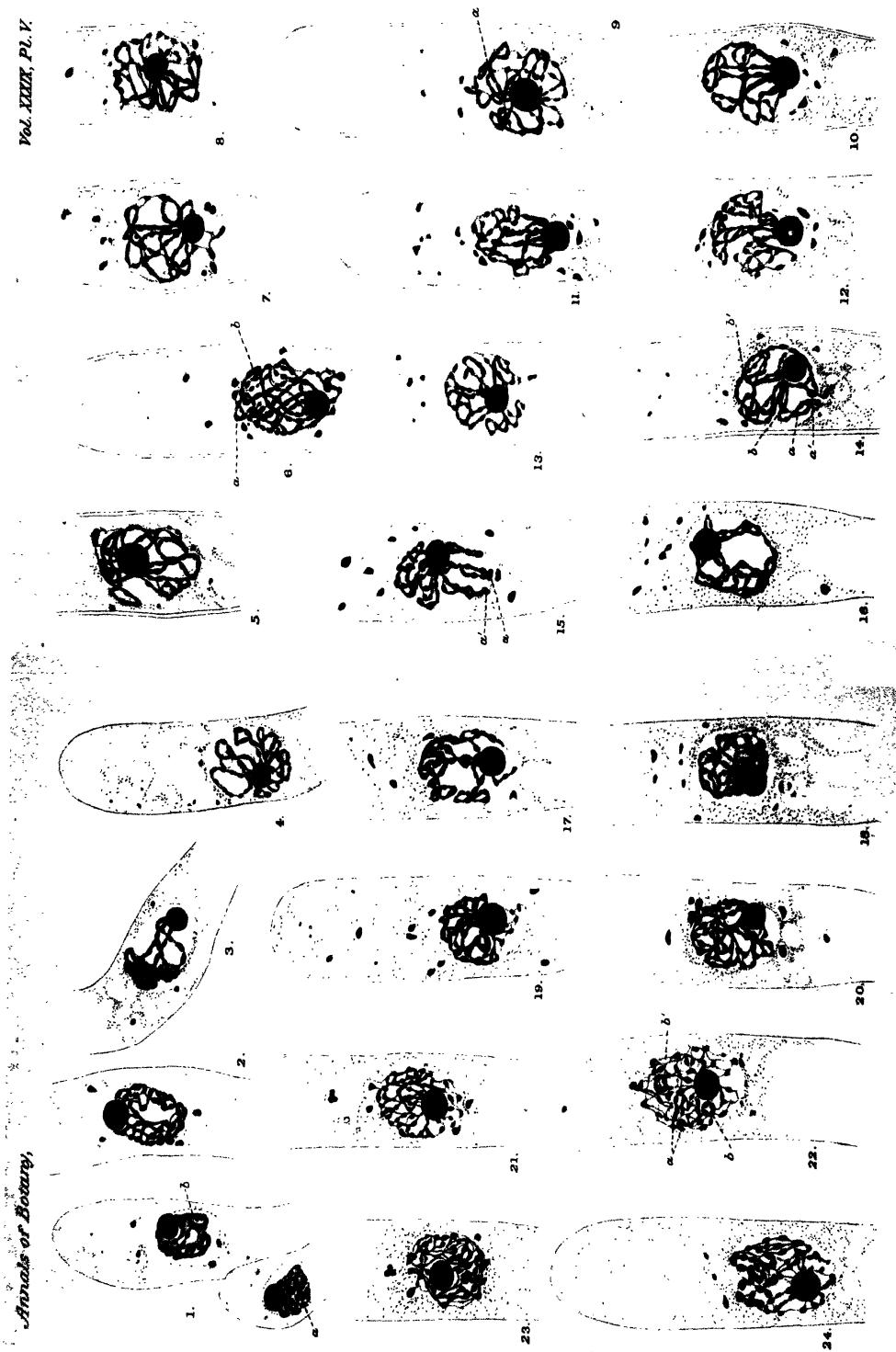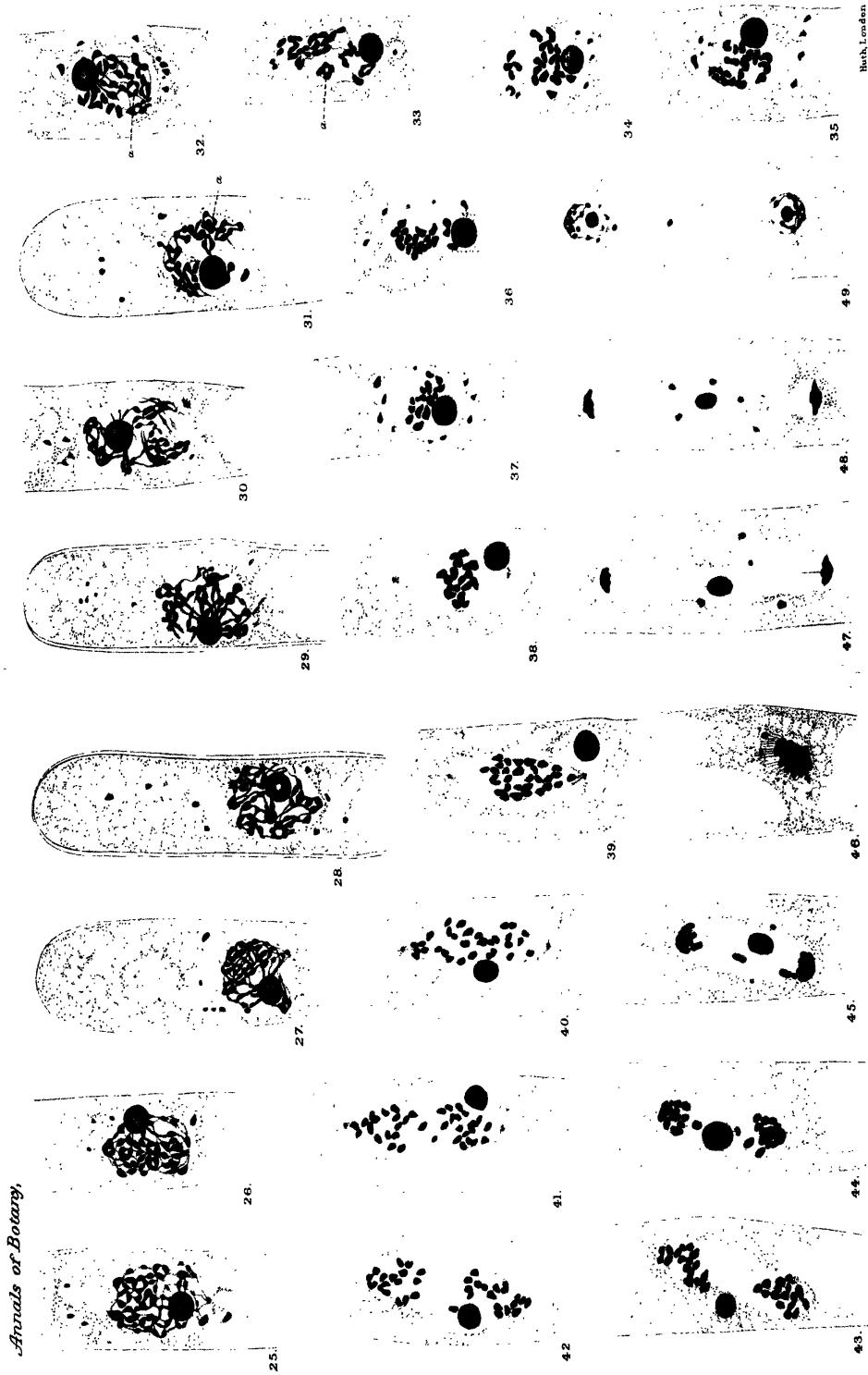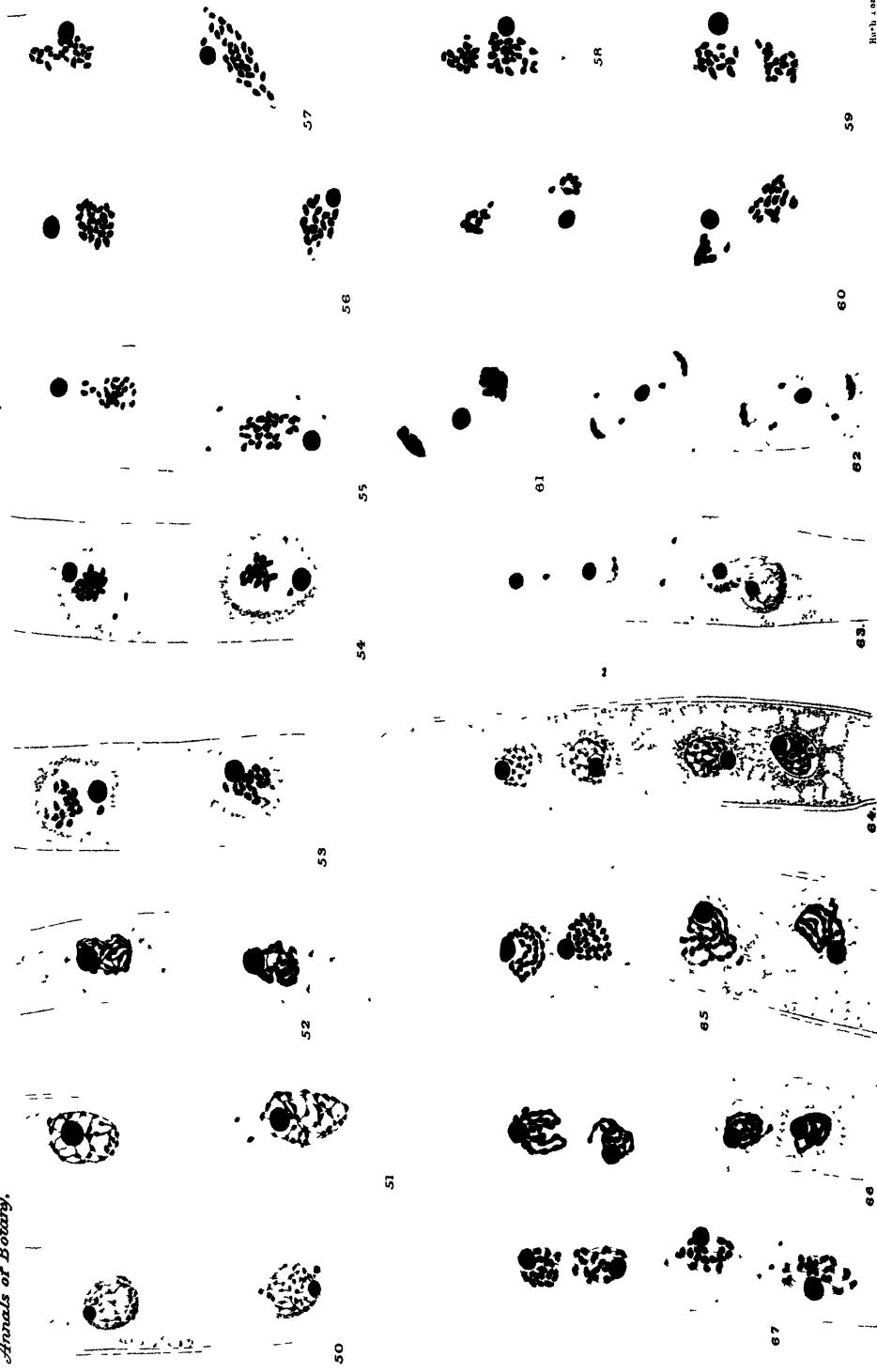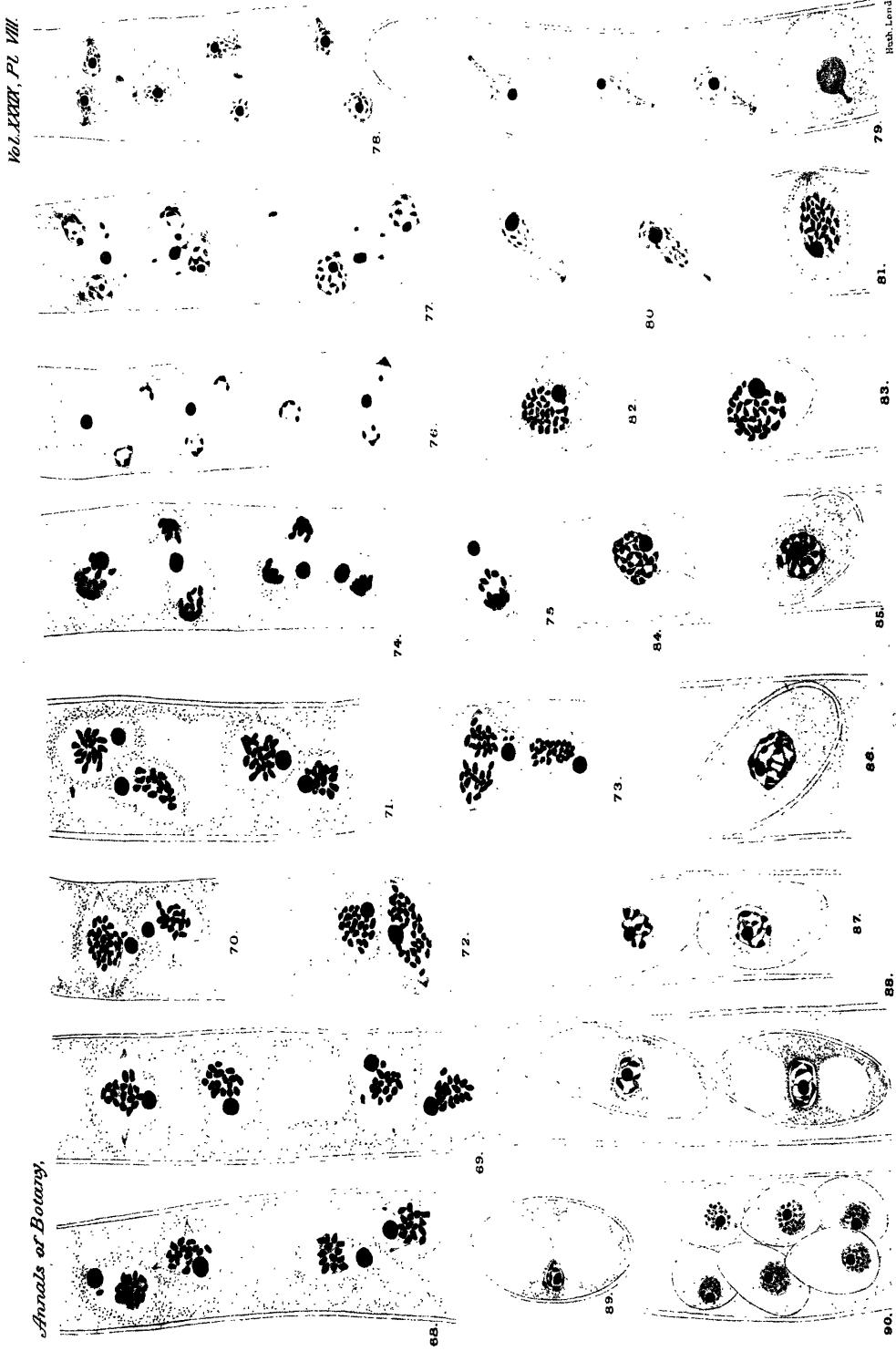
Notes on the Soft Rot of Cotton Bolls in the West Indies caused by Phytophthora.

BY

J. C. HOPKINS, B.Sc.

With seventeen Figures in the Text.

THE Soft Rot of cotton bolls, up to the present only reported from the West Indies, is a disease which, under certain conditions, may assume proportions of decided economic importance. It was first reported from Montserrat by Robson (1) in March, 1911, on a variety of Sea Island cotton known as Rivers; unopened green bolls with blackened tips were very common, but only bolls near the ground were diseased. In 1917 Nowell (2) described a disease of cotton bolls in St. Vincent in which the wall of the boll turned brown and was covered with a glistening powder or white cottony envelope, and both this and the Montserrat disease were found to be due to *Phytophthora*. Nowell further remarks that although a spell of dry weather may check the disease, it is manifest again after two or three days of rain, whilst Robson mentions in his report a particularly wet season. It is now known that heavy or prolonged rain is a necessary condition for the spread of the soft rot, and it is presumed that infection is brought about by motile zoospores being splashed from the soil on to the lower bolls of the cotton plant (3). Infection usually takes place at the tip, or, at least, the rot is first visible at this end of the boll.

Robson (1), in 1911, reported considerable resistance to this disease in the Heaton variety of cotton, which is not typically West Indian Sea Island in constitution, having a very heavy boll, large and coarse leaves, and, strangely enough, throws vigorous laterals and is not a tall grower, thus rendering itself more open to fungus attack. It is said to have been derived from a cross with Upland cotton, and this point is interesting, since, as will be seen later, the writer has found high powers of resistance, amounting under field conditions to immunity, in Upland varieties of cotton. Whether this character is genetic or merely environmental it is difficult to say, because, when grown in St. Vincent, the Heaton cotton was found to be exceedingly

susceptible to soft rot ; but this cannot be said to be due to change in climatic conditions alone, since the St. Vincent *Phytophthora* is a different species from that reported from Montserrat. The latter was isolated by Miss E. M. Wakefield in 1920, and cultures have produced oospores which are of the same size and development as in *P. parasitica*, Dastur (6). The St. Vincent species which was isolated by Ashby in 1921–2 from St. Vincent bolls is quite a distinct form, and cannot be distinguished in culture from the coco-nut bud-rot form (5), which he considers to be *P. palmivora*, Butler ; *P. Faberi* on cacao is apparently a strain or variety of this species. Ashby has also recently isolated a *Phytophthora* from Sea Island cotton bolls in Trinidad, which closely resembles the Montserrat form in growth and asexual reproduction.

The action of these three forms, which may be termed the St. Vincent, the Montserrat, and the Trinidad, on different varieties of cotton is the sub-ject of the present paper. It will be convenient to first describe the inocula-tion experiments and the technique employed, and then to discuss some associated physiological phenomena. Advantage was taken of the fact that cotton bolls, if placed in a damp chamber, continue to grow for some days after they have been picked from the plant. Accordingly the following experiments were carried out.

About half-grown bolls were chosen and the bracts removed to lessen the possibilities of external contamination. They were thoroughly washed with soap and water, dipped for fifteen seconds into 95 per cent. alcohol, to remove air from the surface, and placed in a 0·1 per cent. aqueous solution of mercuric chloride for five minutes; the cut ends of the stalks were covered with paraffin wax and the bolls were washed in three successive jars of water, which had been sterilized in the autoclave, remaining in each jar for five minutes. They were then suspended, tip downwards, by means of sterile cotton threads inside large-stoppered bell-jars on ground-glass plates, both of which had previously been washed with mercuric chloride solution. Petri dishes, each containing a suspension of motile zoospores of one form of *Phytophthora* in 20 c.c. of sterile water, were placed one in each jar, and by loosening the stoppers of the bell-jars the bolls were lowered, by means of the cotton threads, so that the tips were immersed in the liquid. The bell-jars were covered with dark brown paper so that only a small amount of diffused light could enter at top and bottom, and the bolls were allowed to remain thus for forty-eight hours. They were then drawn up to about the centre of the bell-jar and examined from day to day. Control bell-jars were in all cases used, being identical with those described except that sterile water was substituted for the zoospore sus-pension.

In his report on the St. Vincent disease, Nowell mentions that signs of soft rot were observed after two or three days of heavy rain, so that under

the very favourable conditions in the bell-jar, a blackening of the boll should be observed in the same or a shorter period of time. This was found to be the case with certain varieties of cotton, and these are considered as susceptible. Other varieties exhibited a rot after varying periods of time, more or less constant in each variety, but any boll which showed no sign of blackening four days after lifting from the dish was considered to be either highly resistant or totally immune under field conditions. Certain interesting physiological characters were brought to light by these resistant varieties and will be dealt with later. In making zoospore suspensions it was found necessary to adopt the following procedure, since the St. Vincent *Phytophthora* produced sporangia abundantly on culture media on which both the Montserrat and Trinidad strains bore very few or none at all. It was observed that by placing a small piece of aerial mycelium of either the Montserrat or Trinidad fungus in sterile water in a sterile Petri dish and leaving them overnight a copious production of sporangia was obtained in the morning. By draining off the water, replacing by more aerated sterile water and leaving the Petri dish near an open window, a good discharge of zoospores took place within ten minutes. Under the same conditions the St. Vincent form would produce a second crop of sporangia during the night and a corresponding discharge of zoospores could be obtained in the morning.

In describing inoculation experiments the commercial names under which the various cottons are marketed will be used in order to avoid confusion which may arise over questions of taxonomy ; [1] the three forms of *Phytophthora* will be spoken of as St. Vincent, Montserrat, and Trinidad.

In a series of trial experiments it was noticed that St. Vincent, in all cases, was more virulent than either of the other two forms. In one experiment in which old cultures (over a month) were used, no infection was brought about by Montserrat or Trinidad even on the most susceptible varieties, whilst St. Vincent not only caused a rot on these bolls, but also infected a somewhat resistant type.

Bolls of Cauto, Pima, Sea Island, Lone Star, Durango, and Acala were experimented with, but it was found that using more than four bolls in one bell-jar was inconvenient for manipulation and unfavourable for the growth and metabolism of the bolls. Different combinations of the several varieties of cotton were employed and all gave similar results for each variety.

When Sea Island, Durango, Acala, and Lone Star bolls were used, in

[1] *Commercial Name.*	*Group.*	*Systematic Name.*
Cauto	Brazilian	*Gossypium braziliense* var. *apospermum.*
Pima	Egyptian	*G. peruvianum* var.
St. Vincent Sea Island	Sea Island	*G. barbadense* var. *maritimum.*
Durango Acala Lone Star	American Upland	*G. hirsutum* vars.

two days after withdrawal from the zoospore suspension a rot was observed in the Durango boll in the St. Vincent jar and aerial mycelium was visible on the Sea Island. On this variety copious sporangia were produced in four days and cell separation took place in the boll wall. The Durango boll was completely rotted by this time, but cell separation did not appear to have taken place so rapidly as in Sea Island. The boll wall was firm, and hand sections could be cut with ease, whilst it was almost impossible to cut sections of the Sea Island boll wall.

Aerial mycelium was developed on the Sea Island bolls in the Montserrat and Trinidad jars in four days and a few sporangia in five days. Cell separation was complete by this time.

In all bolls that became infected the rot had penetrated to the seed, in which the presence of *Phytophthora* mycelium could be detected; the lint only became stained after the seed had been attacked, i. e. after the fungus had attacked the ' root ' of the hair. St. Vincent and Montserrat sporangia were observed in the lint, and they germinated conidially after being left in a damp chamber for twenty-four hours, but when placed in sterile water in a watch-glass near an open window they discharged zoospores within fifteen and thirty minutes respectively. The Trinidad strain developed sporangia in the lint only after it was allowed to lie in water for twenty-four hours. This reluctance to produce sporangia by Trinidad is worthy of note, since it appears to be a fairly constant character and the only observed point in which it differs from Montserrat.

To test the susceptibility of Pima cotton, an Egyptian variety of the same group as Sea Island, bolls of Cauto, Pima, and Lone Star were used. Pima, as expected, showed a rot in two days in all three bell-jars. At the end of four days complete cell separation in the boll wall had taken place, the seed was attacked and rotted, and the lint stained. Also aerial mycelium was in evidence on the surface of the boll, sporangia being produced by St. Vincent and Montserrat and a few by Trinidad. Sporangia were only observed in the lint of bolls infected by St. Vincent.

Lone Star was completely resistant to the fungus attack and Cauto to Montserrat and Trinidad; St. Vincent, however, brought about a rot of Cauto, but only after fourteen days, and then no cell separation was evident. This infection must have occurred during the time of immersion of the bolls in the zoospore suspension, as they had not been in contact with any other source of infection since being withdrawn. On examination it was found that the seed and lint had been attacked, *Phytophthora* mycelium being present in sections of the seed, but the lint was unstained. Hence it is natural to conclude that the fungus could not have entered the seed more than four days previously, otherwise the lint hairs would have been killed and stained (cf. Pima var.). The probable explanation of the prolonged period before the rot becomes evident would therefore seem to be that the

fungus is capable of establishing itself in the boll wall, and either forcing a way through the tissues extremely slowly, or else lying dormant until the damp atmosphere inside the bell-jar so decreased the physiological resistance of the tissues as to allow an easy passage for the hyphae or brought about autolytic cell separation. This point will be further dealt with in discussing the physiological relations between the host and the parasite.

It may be remarked that in the above experiment a rot appeared at the *stalk* end of the Lone Star boll in the Montserrat jar after five days. This rot was due to *Diplodia* sp., and no evidence of *Phytophthora* could be found ; the writer therefore concluded that it was due to external contamination which might have been picked up in the course of setting up the experiment. It may be mentioned here that, although every precaution was taken, yet several experiments were rendered useless through contamination by species of *Colletotrichum, Diplodia,* and *Fusarium,* the infection probably taking place whilst attaching the cotton threads to the boll stalks, since in all cases the rot started at the stalk end of the boll and so could be distinguished from *Phytophthora* tip infection.

An experiment was made to observe the comparative susceptibility of Durango and Sea Island bolls to the three forms of *Phytophthora*. St. Vincent caused a rot of both ; Montserrat and Trinidad infected Sea Island within three days ; after five days Durango in the Montserrat jar showed a blackening of the boll wall which was due to *Phytophthora*. In the next experiment the effect of prolonged immersion of the bolls in the zoospore suspension was studied. Bolls of Sea Island. Durango, Acala, and Lone Star were chosen as representing susceptible, slightly resistant, and very resistant varieties. About one-eighth of the surface of the bolls was allowed to remain immersed for four days, and the results were somewhat striking.

St. Vincent effected a complete rot of the Sea Island and Durango bolls in three days and Acala in seven days, whilst at the end of that time Lone Star had a peculiar brownish-red rot below the surface of the liquid, with a bright red circle on the water-line. When removed from the water the rot progressed for rather less than two days and then stopped. The boll was allowed to remain for another two days in the bell-jar, but no further developments were observed. A careful examination revealed the presence of no organism, yet the rot had penetrated to the seed.

Sections of the boll wall across the limit of rot showed cells with contents broken down and exhibiting a bright red colour, the cell-walls swollen and intercellular spaces filled with fluid (Fig. 2), in marked contrast with the healthy tissue in which the intercellular spaces were filled with air, and appeared as dark patches throughout the section (Fig. 1). The margin of the affected tissue could be distinctly seen owing to its brilliant red colour in contrast with the normal green tissue beyond. Farther back cell separation began (Fig. 3), and near the tip of the bolls was quite complete. The

liquid, which originally contained the zoospores, assumed a pale red colora-
tion, the pigment being completely water-soluble and having presumably
diffused from the broken-down cells of the boll wall.

Corresponding results were obtained in the Montserrat and Trinidad
jars. Sea Island was alone infected by *Phytophthora*, and that within three
days, aerial mycelium appearing on the fourth. The remainder of the bolls
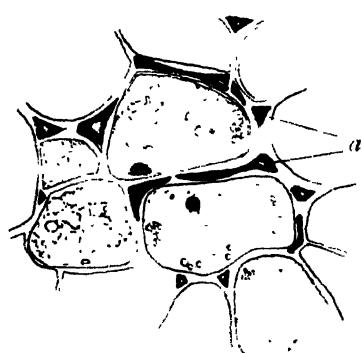
FIG. 1.   FIG. 2.

FIG. 1. Tangential section of boll wall, showing healthy tissue. *a*, air-filled intercellular
spaces.

FIG. 2. Tangential section across rotted area, showing diseased tissue (*s.*, intercellular
spaces filled with liquid.) Cells with broken-down contents, giving characteristic appearance
of red rot.
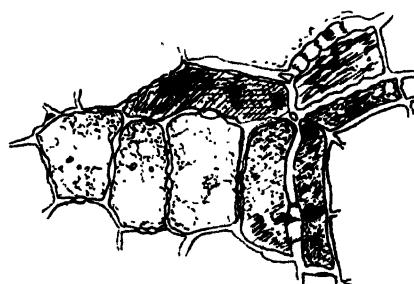
FIG. 3. Red rot : enzymic action on cell-walls and cell-contents. × 360.

showed a red rot which appeared to be identical with that in the St. Vincent
jar, except that the rot had progressed farther and consequently the liquid
in the dishes had assumed a more brilliant eosin-red coloration, more
intense in Montserrat than in Trinidad. In five days the Durango boll in
the Montserrat jar showed signs of *Phytophthora* infection ; but Acala and
Lone Star bolls effectively resisted the fungus. The bolls in the control
bell-jars, though immersed for the same length of time, showed no signs of

rot, but on the contrary were perfectly healthy, so that the red rot must have been due to action on the part of the fungus, i. e. was enzymic.

In another experiment the resistance of Cauto was again demonstrated by employing bolls of Cauto, Pima, Acala, and Lone Star, and it was found also that Pima exhibited highest susceptibility; Cauto showed a delayed rot after sixteen days by St. Vincent, but not by Montserrat nor by Trinidad, whilst Acala and Lone Star were totally resistant.

The table which follows shows the order of the resistance of the varieties tested to *Phytophthora* rot.

TABLE OF RESISTANCE.

Variety of Cotton	St. Vincent.			Montserrat.			Trinidad.		
	Susceptibility.	Minimum time for infection in bell-jar.	Under field conditions.	Susceptibility.	Minimum time for infection in bell-jar.	Under field conditions.	Susceptibility.	Minimum time for infection in bell-jar.	Under field conditions.
Sea Island	+	2 days	very susceptible in wet climate	+	2 days	very susceptible in wet climate	+	2 days	very susceptible in wet climate
Pima	+	do.	do.	+	do.	do.	+	do.	do.
Durango	±	3–4 days	probably susceptible			doubtfully resistant	−		doubtfully resistant
Acala	∓	7 days	resistant	−		resistant	−		resistant
Cauto	−	12 16 days	resistant	−		resistant	−		resistant
Lone Star	−		probably immune	−		probably immune	−		probably immune

NOTE.—Young bolls were found to be more resistant than those more mature, and no infection was brought about on any Upland bolls less than half grown. Sea Island and Pima bolls, however, appear to be susceptible in all stages of development.

The positive and negative signs in the susceptibility column are used as standards of comparison. Thus + represents a variety of cotton in which infection took place in the bell-jars in every case, no matter what period of time elapsed before the rot became evident. When ± is used, it represents occasional infection in a short time and ∓ after a prolonged period; whilst − shows no infection was obtained.

Microscopic. In all the diseased bolls from the infection experiments, the course of the fungus was followed through the tissues and its action upon them studied.

The fungus enters the boll wall by the germ-tube penetrating the epidermis, or sometimes entering through a stoma. The hyphae grow through the intercellular spaces of the tissue (Fig. 4), but later become intracellular (Figs. 5 and 6). It is only in the older diseased tissue that the latter condition is found, and it is evidently brought about by late-formed lateral branches from the intercellular hyphae in tissues already dead or dying. The fungus passes through the boll wall and enters a loculus, in which it appears to attack the seed first, because although it is possible to distinguish the mycelium amongst the lint hairs, yet no staining of the lint
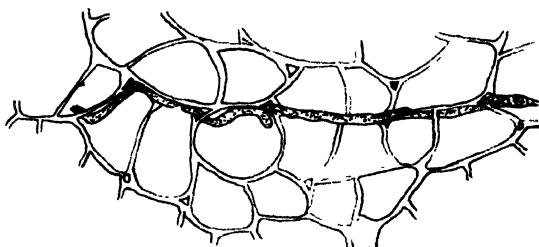
FIG. 4.   Hypha in intercellular spaces.   × 360.
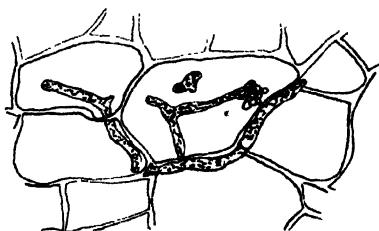
FIG. 5.

FIG. 6.

FIG. 5.   Intracellular hyphae.   × 400.
FIG. 6.   Haustorium-like process, *h.*, in cell of boll wall.   × 360.
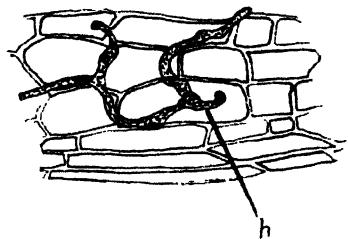
occurs in newly diseased bolls. In bolls that have been infected for a longer period (about five days) stained lint is plentiful and hyphae can be observed *in* the lumina of the hairs (Figs. 14 and 17).

It occurred to the writer that it would be interesting to observe the behaviour of zoospores on the epidermis of the boll, and so a modified hanging-drop culture was prepared as follows :

A small cell about 2 mm. in diameter was made on a cover-slip by painting a circle of vaseline with a fine camel-hair brush. Into this was placed a drop of sterile water containing motile zoospores and the whole covered with a piece of epidermis stripped from the lower side of a cotton leaf. The cover-slip was mounted in the usual way for hanging-drop cultures, and the behaviour of the zoospores could then be easily followed,

since the thin epidermis offered no obstacle to the passage of light. The zoospores swam about apparently in an aimless fashion for about five minutes, then settled down suddenly, without any sluggish movements, and rounded off. Within five minutes the margin of the spore became more refractive, due apparently to the formation of a wall, and about twenty minutes later germination began by a small papilla being pushed out from the spore (Fig. 9, *a*). The wall of the papilla was thinner than that of the spore and appeared to be the spore wall stretched. The germ-tube
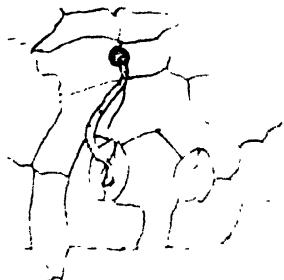
FIG. 7.

FIG. 8.

FIG. 7. Germ-tube entering a stoma. · 360
FIG. 8. Germ-tube on the surface of the epidermis of the leaf. No penetration. × 360.

a *b* *c* *d*
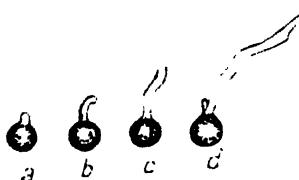
FIG. 9. Germination of a spore.

remained thus for ten minutes, when movement was observed in the proto-plasm of the spore and the tube began to grow out. Again, the growing tip had a wall much thinner than the remainder of the tube. Growth continued for about ten to fifteen minutes and again ceased, when the germ-tube measured 6.3 μ in length. Another period of rest followed, during which the tube tip thickened and a streaming movement was noticed in the protoplasm; later growth continued in the same fashion. Finally, after a period of rest, the tip of the germ-tube appeared to be blown out as a bladder (Fig. 9, *b*), not in the original direction of growth, but alternately first to one side and then to the other (Fig. 9, *c* and *d*). No penetration of the epidermis was noticed and only one case of the germ-tube passing through a stoma (Fig. 7). The majority of hyphae grew over the surface

of the epidermis (Fig. 8), and were observed as a tangled web of mycelium after twenty-four hours. The stimulus responsible for inducing the fungus to enter the boll was apparently absent in the above experiment, but by substituting tangential sections (about three cells in thickness) of the boll wall for the leaf epidermis, and repeating the experiments, two cases of penetration of the boll epidermis were observed (Figs. 10 and 11). In one case the hypha entered after passing over a stoma, and forced its way through the centre of a cell, whilst in the second case entrance was effected
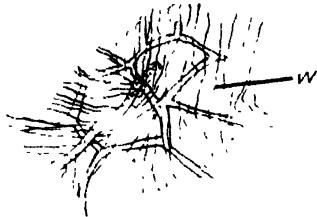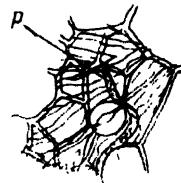
FIG. 10.   FIG. 11.

FIG. 10.   Penetration between two cells of the boll epidermis.   × 430.   *w.*, wax incrustatio on boll.

FIG. 11.   Penetration through a cell of the boll epidermis.   × 360.   *p.*, point of entry.
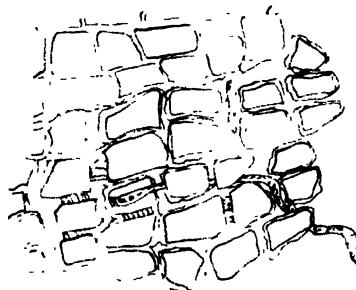
FIG. 12.   Action of hyphae in tissues of the boll wall (Sea Island and Pima varieties).   Semi-diagrammatic.

by forcing a passage between two epidermal cells. It is perhaps allowable to conclude from this that penetration of the boll epidermis was in response to some stimulus which was present in the section of the boll and not in the stripped lower epidermis of the leaf.

Having entered the host, the hypha passes through the intercellular spaces of the boll wall, causing a break-down of the cells as it progresses. After a while the cell walls become swollen, at first irregularly with numerous constrictions, and eventually become quite homogeneous in structure, staining a pale violet colour with chlor-zinc-iodine (Fig. 12). The walls, after being weakened in this way, are unable to resist the hyphae, which force their way into the cells, causing a complete disintegration of the contents to reddish or reddish-brown products.

A distinction must here be pointed out between resistant and susceptible varieties of cotton. As mentioned in an earlier part of the paper, susceptible varieties showed a complete cell separation in the boll wall two days after infection, whilst a corresponding break-down of tissue is not observed in resistant bolls within the same period of time. Microscopic examination revealed the fact that the swelling of the cell wall was common to both types, and appeared to take place in the same way, but complete cell separation with solution of the middle lamella was considerably delayed in Upland and Cauto bolls. It follows therefore that the passage of the hyphae is obstructed to a great extent by the non-disintegration of tissue, and this must to some extent be responsible for the resistance to the rot exhibited by these cottons. Indeed, it is quite reasonable to suppose that
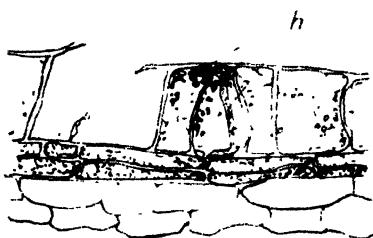
FIG. 13.

FIG 14.

FIG. 13. Mycelial aggregate beneath epidermis of the seed-coat.   × 360.   *h.*, hyphae.
FIG. 14. Penetration of lint hair.   × 520.
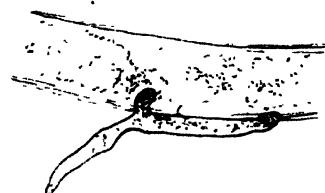
this may be a limiting factor in varietal susceptibility, especially when it is remembered that infection (i. e. penetration) can take place within two days, but that boll rot (i. e. disintegration and cell separation) may be delayed for as long a period as sixteen days.

Infected tissues were examined for haustoria. Dastur (6) figures haustoria-like processes in cells of castor infected by *P. parasitica*, and the writer has found similar structures in cells of the boll wall (Fig. 6), but agrees with Dastur that these have more the appearance of hyphal branches than of true haustoria, as intracellular mycelium was common in older diseased tissue. The case is, however, different in considering infection of the cotton lint. The mycelium grows amongst the lint hairs for some time without penetrating them, but attacks the seed. Examination of diseased bolls in a more advanced stage showed numerous cases of hyphae in the lumina of the lint hairs, and Fig. 14 shows what the writer regards as a true haustorium which has just penetrated the cellulose wall. What appears to be an appressorium-like structure at the hypha tip is in reality the optical effect produced by the hypha turning and growing at right angles to the plane in which the drawing is figured.

In the seed the action of the fungus is similar to that in the boll wall.

Hyphae penetrate to the endosperm and cotyledons, causing a break-down of the cells to a brown pulpy fluid, the testa alone retaining its firmness. Later still the lint hairs die and become stained a deep brown also. The method by which the hyphae grow out from the host tissues corresponds to that generally described for *Phytophthora* (3). Fig. 13 shows a mycelial aggregate beneath the epidermis of the seed-coat. This apparently increases in size and eventually ruptures the epidermis, although this stage was not observed by the writer. Tangential sections showed numerous branched hyphae growing out from stomata on the bolls infected with St. Vincent

FIG. 15.   FIG. 16.   FIG. 17.

FIG. 15.   Hyphae growing out from a stoma   x 360.
FIG. 16.   Solution of cell-wall   s , partly dissolved wall.   x 520.
FIG. 17.   Hyphae in lint hair.   x 520.
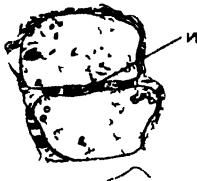
(Fig. 15), but in no cases were hyphae observed to grow from stomata of bolls infected with Trinidad or Montserrat, escape being accomplished by rupture of the epidermis or by forcing a passage between cells.

It is now convenient to draw a comparison between the rot brought about by the fungus in the tissue of the boll and the red rot already described, in which no organism could be detected and which was presumably due to enzymes.

The macroscopic appearance of bolls affected with these two rots presents a strong contrast. The fungal growth causes a typical blackening of the boll tip which progresses throughout the tissue and is complete in from two to three days; the second appears as a light brownish-red rot with a bright red margin, which does not spread through the boll, but is arrested soon after the boll is removed from the inoculated liquid. Microscopic examination, however, shows solution of the cell-walls, first as a swelling with numerous constrictions, and eventually a complete separation as described already for the *Phytophthora* rot and as shown in Figs. 3, 12,

and 16. The effect upon the seed and lint is also similar, in that complete disintegration is brought about, resulting in stained and ultimately rotted lint and a completely disorganized seed. It is evident, therefore, that the fungus excretes an enzyme capable of causing disorganization of the boll tissues and cell-wall solution. The suggestion that it is a cellulose-dissolving enzyme is further substantiated by the fact that germ-tubes of sporangia germinating conidially, or of undischarged zoospores germinating *in situ*, grow through the sporangial wall, which is of pure cellulose. The papilla, the plug of which, according to Dastur (6), consists of pure callose, is never punctured, although the germ-tubes may form a rosette round it. The germ-tubes of undischarged zoospores may, however, grow out through the open papilla.

Fig. 16 shows a tangential section of a boll wall near the margin of the red rot. Every stage in cell separation can be observed, from the initial swelling of the wall to its complete disintegration. When stained with chlor-zinc-iodine, the intensity of the violet colour in the cell-walls varies inversely with the degree of separation that has taken place, the less affected walls showing the deepest coloration. As the degree of staining would naturally vary with the concentration of cellulose in the cell-wall, it is reasonable to conclude that a cellulose-dissolving enzyme is concerned with cell separation in the boll.

Control. Possibilities of control by spraying with Bordeaux mixture have been demonstrated by Harland in St. Vincent during an attack by the disease upon his boll-shedding experimental plots, but this method has not been tried extensively in the fields, although theoretically it should prove effective (7).

SUMMARY.

1. At least two species of *Phytophthora*, one of them apparently having two strains, are responsible for the soft rot disease of cotton bolls in the West Indies.

2. Varying powers of virulence have been established for these fungi and details of the relative susceptibility or resistance of six varieties of cotton described.

3. It has been shown that St. Vincent, Montserrat, and Trinidad forms of *Phytophthora* excrete a cellulose-dissolving enzyme which can cause a rot and cell separation in the absence of the fungus mycelium from the host tissue.

4. The behaviour of zoospores, germination of the resting spore, and growth of the germ-tube are described, and details of the technique employed to illustrate their behaviour on the surface of an epidermis are furnished.

5. Penetration of the boll and the passage of the fungus through the host tissues are followed and the significance of certain structures discussed.

6. The rot resulting from growth of the fungus in the tissue and that due to enzymes are compared and the similarity between the two pointed out.

7. The possibility of control by spraying with Bordeaux mixture is indicated.

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The Growth of the Cotton Plant in India.

I. The Relative Growth-rates during Successive Periods of Growth and the Relation between Growth-rate and Respiratory Index throughout the Life-cycle.

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With eight Figures in the Text.

CONTENTS.

I. INTRODUCTION.

K IDD, West, and Briggs (1 and 2) have carried out an elaborate investigation of the quantitative analysis of growth in *Helianthus*. On the suggestion of Dr. F. F. Blackman, it was decided to apply their methods of work to tropical plants with a view to evaluate quantitatively the internal and external factors concerned in growth. Such evaluation is especially

interesting in the case of a plant of agricultural importance, so Cotton was chosen as the subject of investigation.

Interest in this branch of knowledge was stimulated by the application by V. H. Blackman (3 and 4) of the 'compound interest law' to plant growth. Blackman represents the growth of a plant, as measured by the increase in its dry weight, as similar to capital increasing continuously at compound interest; the initial capital is the dry weight of the seed or the seedling at the beginning of the experiment, and the rate of interest, the rate of growth per unit dry weight during any particular period of the life-history of the plant. The rate of interest calculated in this way is termed by Blackman the 'efficiency index' of dry-weight production, or the 'economy constant' of the plant. Kidd, West, and Briggs (5) have questioned the physiological significance of the efficiency index. They point out that the rate at which dry matter is added does not necessarily remain uniform even for short periods in the life-history of the plant. It must be admitted that, as Blackman claims, plants do to a large extent add new material on the compound interest principle. Hence Blackman's formula[1] can be used as the nearest approximation to indicate 'relative growth-rates' during the successive periods of growth for purposes of comparison. Kidd, West, and Briggs themselves, having come to the conclusion that in the early part of the plant-growth the increment of dry weight and leaf area is more or less exponential, whilst later it approaches to linear, use a slightly modified formula[2] (6), on the exponential basis, to express growth-rate. They define their relative growth-rate, R, as the average rate of increase in dry weight per unit dry weight per week (expressed as a percentage). There is no fundamental difference mathematically between the two formulae if time t is eliminated from Blackman's formula by taking successive observations at a definite interval of time, the shorter the better. In the following series of investigations, the formula adopted by Kidd, West, and Briggs has been used to express weekly relative growth-rates, the weekly rates being calculated wherever observations are not taken regularly every week.

II. Methods of Investigation.

The experiments described below were designed with a view to obtain a comparison of:

(*a*) The relative growth-rate curves of plants grown in different periods of the year.

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[1] The formula used by Blackman is $W_1 = W_0 e^{rt}$, where W_1 is the dry weight of the plant at the end of the time t, W_0 the initial seedling or seed weight, r the rate of interest, and e the base of natural logarithms.

[2] Their relative growth-rate, R, is calculated by the formula: $\dfrac{R}{100} = \log_e W_2 - \log_e W_1$, where W_2 and W_1 are the dry weights at the end and the beginning of the week, respectively, and e the base of the natural logarithms.

(*b*) The relative growth-rates with variations in the leaf-weight ratio and the leaf-area ratio.

(*c*) The relative growth-rates on the one hand and variations in the respiratory indices of the entire plant and of its parts throughout the life-cycle on the other.

While it is intended to measure growth-rate and respiration in plants grown in different seasons of the year, the results discussed below were obtained on the seeds germinated on the 14th of May 1923 (first series), on the 6th of June (second series), and on the 15th of July (third series). The relative growth-rates and the percentage dry weights of the roots, stem, and leaves were measured in all the three series. The course of the respiratory index was measured only in the first series, while measurement of the area exposed by the leaves during successive periods of growth was taken in the second and the third series.

The atmospheric weather conditions were very severe during the month of May and the greater part of June. The maximum temperature of the atmosphere in shade varied from 100° F. to 113° F. till the last week of June, when the first rains commenced. The atmosphere during this period was dry, the humidity varying from 35 per cent. to 40 per cent. There were also occasional hot winds, especially between the 6th and the 15th June, which were very unfavourable for growth. There was bright sunshine during the whole of this period. The conditions became more favourable for growth after the beginning of the rains. Though the first rains fell in the fourth week of June, the actual rainy season with cloudy weather did not begin till after the second week of July. Up to the commencement of the rains, the plants had to be kept in a cool, shady place during the hottest parts of the day, exposing them to direct sunlight only in the mornings and evenings. Excepting the unavoidable variations in the weather conditions all the plants of the same series were given identical treatment. No attempt is made in this communication to correlate the amount of growth with the external factors, the object being only to trace the form of the relative growth-rate curves in plants grown during different periods of the year and to compare the curves with all other observations made in the respective series.

Selected seeds of the variety *roseum* of cotton were used for all the series. They were sown separately in pots. and only plants which germinated on the same day in each series were kept for investigation. Dry weight and other observations were recorded every time by uprooting one or more plants, according to their size. The pots were brought into the laboratory and the plants, along with their roots, were carefully removed by playing a jet of water on the soil. The fresh weight of the entire plant and of its parts separately was taken immediately. The plants were then kept for drying at 100° C. after measuring either the respiration

or the leaf area as the case might be.   For the leaf area all the leaves were carefully detached and traced on a white sheet of paper, taking great care that there was no shrinkage during this process.   The area thus traced was measured in centimetres with the aid of a planimeter.

The respiration was measured in the laboratory at a definite tem-perature, viz. 38° C.   Separate estimations were made for roots, stem, and leaves.   In some cases, especially when the plants advanced in growth, respiration was taken only with a fraction of the fresh weight and the total respiration in each organ was calculated.   The material to be investigated was sealed air-tight in a suitable glass chamber which was immersed in a water-bath kept at the constant temperature.   The chamber was supplied with atmospheric air freed from carbon dioxide, and the carbon dioxide produced during respiration was drawn by an aspirating current through Pettenkofer tubes containing excess of baryta.

Usually three observations were taken at an interval of two hours each after allowing a preliminary of exactly two hours.   Owing to the high tem-perature used, the respiration does not remain constant during successive observations.   This difficulty could not be removed, however, due to want of facilities in this laboratory for keeping constant for a period of eight hours a lower temperature than the atmospheric temperature.   It is possible to obtain absolute initial values of respiration from these data by extrapolating the curves back to zero time, but it is doubtful whether the results obtained in this way can be relied upon for purposes of comparison.   The falling off in successive periods is, however, comparatively slight at this tempera-ture (see Appendix, where the data are given in detail).   It can also be safely assumed that the effect of ' time factor ' due to high temperature is very nearly the same in each organ during successive periods of growth, and wherever there is any serious difference noticed, its significance is indicated below.   The results obtained indicate thus only comparative values during successive periods of growth.   Since no continuous records of the tempera-ture at which the plants were respiring in the open were kept when these experiments were conducted, no attempt is made to deduce absolute values of CO_2 respired by the plant under field conditions.   For purposes of com-parison hourly averages of six-hour results obtained at 38° C. in the labora-tory were reduced to per gramme dry weight.   This is termed the ' respiratory index ', which is compared with the relative growth-rate.   The respiratory index of the entire plant was calculated by taking into considera-tion the relative dry weight of each organ and its respiratory value per hour per unit dry weight.

III. DISCUSSION OF THE RESULTS OBTAINED.

A. Comparison of the relative growth-rate curves of plants grown in different periods of the year.

The relative growth-rates in each series are tabulated in column 3 of Tables 1, 2, and 3, respectively, and are graphically represented against time in Fig. 1.

TABLE 1. *Cotton.—First Series.*

(Germinated on the 14th of May 1923.)

Date.	Days after Germination.	Relative Weekly Growth-rate. Per cent.	Percentage Dry Weight of Different Parts to the Total Dry Weight of the Plant.			Number of Plants examined.	Remarks.
			Root.	Stem.	Leaves.		
1	2	3	4	5	6	7	
22.5.23	9		8.79	31.66	59.55	23	
		60.1					
27.5.23	14		15.5	34.8	49.10	15	
		81.9					
3.6.23	21		17.2	33.6	49.2	13	
		84.0					
10.6.23	28		16.13	33.44	50.43	5	Hot and stormy winds unfavourable to growth.
		0.34					
17.6.23	35		17.21	33.39	49.4	5	
		82.0					
24.6.23	42		15.76	39.04	45.2	3	
		80.4					
1.7.23	49		16.55	30.75	54.7	2	Rains commenced.
		117.02					
8.7.23	56		21.96	22.53	51.51	1	
		16.15					
15.7.23	63		18.54	30.97	50.49	1	Continuously heavy rains commenced.
		26.25					
4.8.23	83		25.95	24.34	49.71[1]	1	Flowering period commenced.
		15.73					
18.8.23	97		20.91	4.58	44.51[1]	1	
		1.02					
9.9.23	119		22.62	2.64	22.26 (23.48 flower and fruit)	2	

The variations in the weather conditions affect the smoothness of the curves considerably. For instance, the cessation of growth between the 28th and the 35th day in the first series and between the 8th and the 15th day in the second series is due to the prevalence of hot winds during the second and the third week of June. Similarly, the secondary increase in the growth-rate between the 43rd and the 50th day in the second series must be due to the particularly favourable conditions for growth, as the rains began definitely during this period. But if these sudden rises and falls

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[1] These represent both the reproductive organs and the leaves, which were dried together by mistake.

are left out of consideration the growth-rate curves show, in general, an initial increase leading gradually or rapidly to a *maximum* which is followed by a steep fall later. The differences observed in the different curves relate merely to the period before the *maximum* increase, according to the duration of the vegetative period. In Cotton the flowering period commences earlier the later the seeds are sown in the rainy season. In the first series, where the flowering period does not commence till the 83rd day after germination, the growth-rate suddenly increases during the first fortnight, and remains practically constant between the 14th and the 49th day, after

TABLE 2.   *Cotton.—Second Series.*

(Germinated on the 6th of June 1923.)

Date.	Days after germination.	Relative Weekly Growth-rate. Per cent.	Percentage Dry Weight of Different Parts to Total Dry Weight of the Plant.			Leaf Area. Per Grm. Dry Weight of the Leaves.	Per Grm. Dry Weight of the Plant.	Number of Plants taken.	Remarks.
			Roots.	Stem.	Leaves.				
1	2	3	4	5	6	7	8	9	10
6.6.23	1		Not observed		...	...	...	110	
		6.7							
13.6.23	8		5.5	6.73	57.77	221.76	128.09	5	Hot and stormy winds unfavourable to growth.
		0.97							
20.6.23	15		9.73	42.41	47.86	276.42	132.29	5	
		52.86							
27.6.23	22		11.93	39.45	48.67	283.02	137.61	5	Rains commenced.
		133.6							
4.7.23	29		12.06	29.55	58.39	341.25	119.27	2	
		104.62							
11.7.23	36		19.65	23.8'	56.55	288.41	163.16	2	
		88.88							
18.7.23	43		15.58	20.53	63.89	227.3	145.23	2	Continuously heavy rains.
		127							
25.7.23	50		12.6	26.67	64.73	200.86	121.98	2	
		19.34							
1.8.23	57		15.77	22.67	61.56	180.82	111.31	2	
		24.27							
8.8.23	64		15.41	28.12[1]	56.47	171.52	96.86	2	Flowers appeared on the plants.
		10.69							
15.8.23	71		17.51	27.12[1]	55.37	157.66	87.33	2	
		5.82							
23.8.23	79		19.25	34.02[1]	46.73	160.67	75.07	2	

which there is a *maximum* increase followed by the usual fall. In the second series the purely vegetative period continues only up to the 64th day, and there is also a corresponding change in the relative growth-rate curve. The constant rate of growth noticed after the initial sudden increase in the first series has here disappeared entirely, and the curve, excepting the secondary rise, which is already explained, is a simple parabola. It is to be

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[1] Dry weights of the reproductive organs are also included in these.
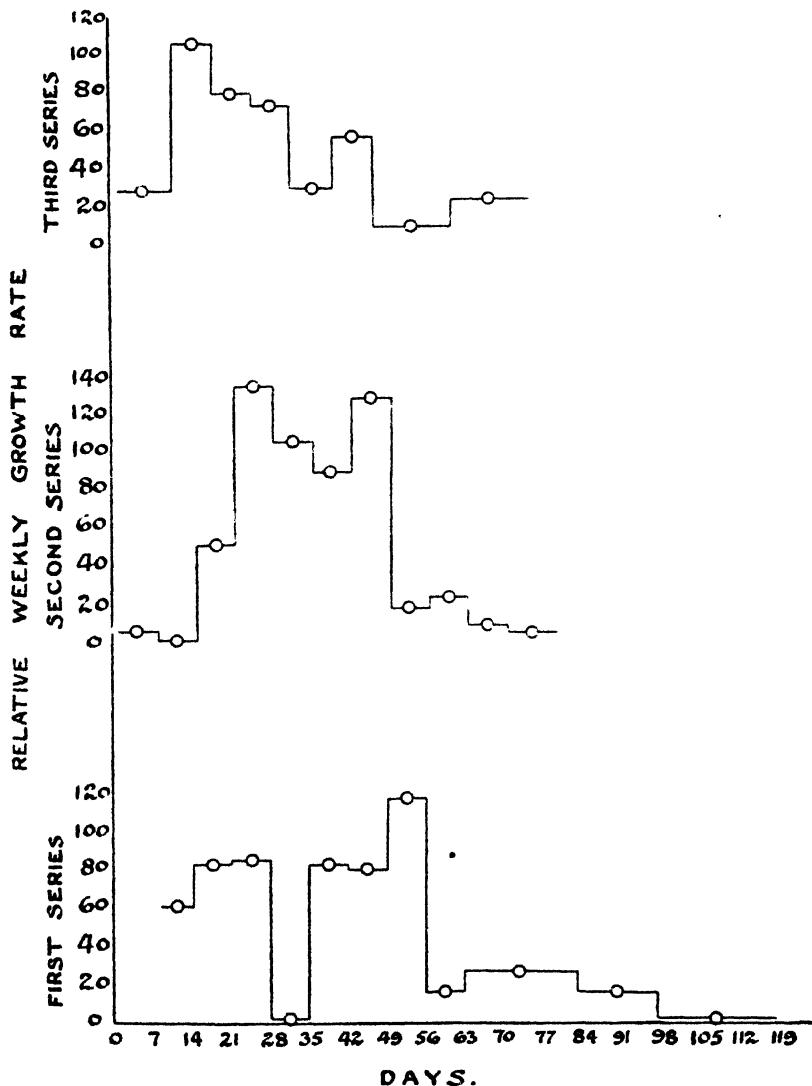
FIG.I

noted here that the *maximum* increase in the first and the second series occurs at about the same period, viz. during the latter part of June and the early part of July, when there were heavy initial showers. In the third series the vegetative period is still shortened, and, though the growth-rate curve is of the same form as in the second series, the *maximum* increase takes place much more rapidly than in the first two series.

Growth-rate was also observed in the particularly short-lived plant,

TABLE 3. *Cotton.—Third Series.*

(Germinated on the 15th of July 1923.)

Date.	Days after Germination.	Relative Weekly Growth-rate. Per cent.	Percentage Dry Weights of Different Parts to Total Dry Weight of the Plant.			Leaf Area. Per Grm. Dry Weight of the Leaves.	Per Grm. Dry Weight of the Plant.	Number of Plants taken.	Remarks.
			Roots.	Stem.	Leaves.				
1	2	3	4	5	6	7	8	9	10
15.7.23	1		...	...	...	...		25	
		28.2							
25.7.23	11		10.06	29.78	60.22	348.57	209.73	20	
		106.24							
1.8.23	18		18.5	28.7	52.8	313.06	156.3	15	
		80.44							
8.8.23	25		16.06	27.04	56.9	238.42	136.17	5	
		73.01							
15.8.23	32		15.79	21.54	62.67	200.28	125.52	5	
		30.15							
23.8.23	40		20.91	23.18	58.91	213.56	119.4	4	
		56.97							
30.8.23	47		16.66	13.12[1]	52.22	202.98	105.98	4	Flower-bud on one of the plants.
		11.60							
13.9.23	61		15.92	40.17[1]	43.91	158.26	68.15	2	
		23.34							
27.9.23	75		24.41	36.16[1]	39.43	139.25	54.71	2	Bolls appeared.

Impatiens, for comparison with Cotton. The results obtained are given in column 2 of Table 4. In Fig. 2 the relative growth-rate curve of Balsam is compared with that of the second series of Cotton grown during the same period of the year. The agreement between the two curves is so close that even the periods of *maximum* increase in the two correspond with each other.

The results described here, taken in conjunction with those obtained by Kidd, West, and Briggs in *Helianthus*, do not lend any support to the existence of a 'grand period' of growth in plants with a continued maximum rate of growth. The *maximum* growth-rate is not sustained for any length of time, but is attained sooner or later according to the duration of the vegetative period of the plant, which varies from season to season. Whether the whole of this variation is to be ascribed to the varying external factors,

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[1] Dry weights of reproductive organs are also included in these.

or a part of it is due to the inherent 'periodicity' of the plant, is a question which can be decided only when a continuous record of all the external factors has been kept throughout the growing period. It will also be

TABLE 4. *Balsam.*

(Germinated on the 10th of June 1923.)

Number of Days after Germination.	Weekly Relative Growth-rate of the Plant. Per cent.	Percentage Dry Weights of Different Parts to the Total Dry Weights of the Plants.			Remarks.
		Roots.	Stem.	Leaves.	
1	2	3	4	5	6
7		...	...	...	The plants were too small to be divided into the three parts.
	25·65				
28		27·37	15·64	56·98	
	156·1				
37		20·59	19·70	59·7	Flowering commenced on the 40th day.
	84·28				
51		25·95	24·18	48·88	
	33·6				
63		26·32	29·32	44·44 [1]	
	13·51				
85		19·94	40·05	34·43 + 4·56 (fruits and flowers)	

FIG. 2
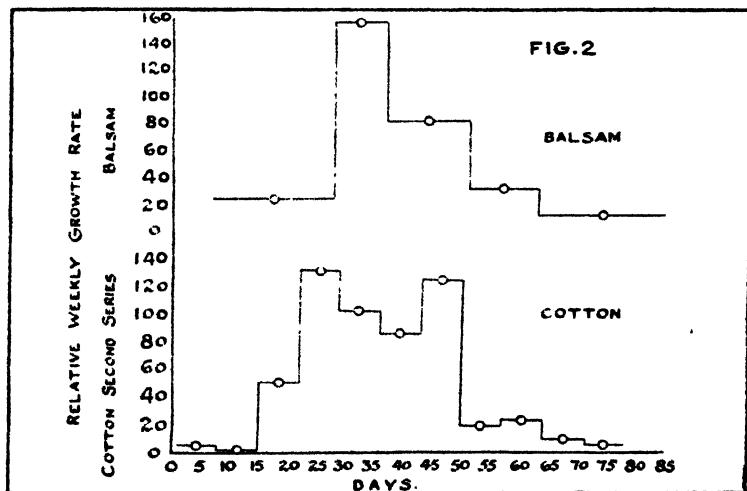
interesting to investigate, by observations on different species and varieties grown during different seasons of the year, whether this is a specific difference distinguishing the two classes of annuals, the short-lived and the long-lived.

[1] The dry weights of reproductive organs are also included in these.

It is worth noting that in all the three series of cotton the flowering period commences at a definite interval after the *maximum* increase, viz. from 27 to 35 days. It appears as if the *maximum* increase in the growth-rate supplies the necessary internal stimulus for the plant to set itself to produce the reproductive organs, the 'latent period' being about a month in the variety studied. If this is a general truth, the *maximum* increase in the growth-rate must have a deep physiological significance, and requires further elucidation. From a purely economic point of view, the longer the period before this stimulus is given the greater is the yield. The ultimate yield of cotton was not recorded in any of the three series. But judging from the absolute dry weights per plant when the growth-rate was farther down on the decreasing side, viz. on the 70th day after germination in each series, it appears that the earlier the crop is sown the greater is the yield. The absolute dry weights per plant calculated for the 70th day after germination are 14·69 grm. in the first series, 13·9 grm. in the second series, and 10·6 grm. in the third series, showing a regular gradation of decreasing values. But sowings on a large scale cannot be made in these tracts as early as is desirable, owing to the late appearance of the rains, viz. during the latter part of June.

B. Comparison of the relative growth-rates with variations in the leaf-weight ratio and leaf-area ratio.

West, Briggs, and Kidd (6) have suggested a number of formulae to facilitate comparison of the assimilatory activity of the leaves with the relative growth-rate of the plant as measured by its dry weight. Of these, their relative leaf growth-rate, R_1, expresses merely the rate at which the leaf area is increasing in a given time per unit leaf area. But the increase in the leaf area is more a function of the unit dry weight of the plant than of the unit leaf area, and it influences in its turn the total dry weight of the plant. Hence their R_1 has very little significance from the point of view of comparing the assimilatory activity of the leaves with the relative growth-rate. The two other formulae are for determining the leaf-area ratio, A, and the unit leaf-rate, E. By leaf-area ratio they mean the ratio of leaf area to dry weight, that is $\frac{L}{W}$, and by unit leaf-rate the weekly rate of increase in dry weight per unit leaf area. On the exponential basis, the latter can be determined by the formula $\frac{dw}{dt} = EL$. It is undoubtedly important to determine both of them in an attempt to evaluate the mutual relations of the assimilatory activity of the leaves and the relative growth-rate of the plant. But they will have to be determined each from independent observations—the leaf-area ratio from the observed data of the area exposed by the leaves during successive periods of growth, and the unit

leaf-rate independently by the assimilatory activity of the leaves under given conditions of environmental factors at different stages of growth. From the formulae which the three authors suggest, the relative growth-rate is merely the product of the leaf-area ratio and the unit leaf-rate multiplied by 100. So long as these two data are not obtained from independent observations, this statement implies that the relative growth-rate, as measured by the dry-weight production of the plant, is merely a function of the area exposed by the leaves and the dry weight produced by the leaf per unit area, a problem which itself is to be investigated, on account of the possibility of several internal factors which control the quantity of dry matter present in the plant at any stage of its life-history.

Further, their attention is concentrated only on the area exposed by the leaves without any reference to the leaf substance as measured by its dry weight. Müller's (7) observations on the assimilatory capacity of the sun and the shade leaves in *Juglans regia* and *Sambucus nigra* are very instructive in this connexion. Müller compared the assimilation per unit area and per unit weight in the sun and the shade leaves in bright sunshine and in shade. When the sun leaves with a thicker leaf structure were exposed to bright sunshine, the assimilation per unit weight of the leaf was less than that per unit area, a result that can be explained by assuming that in bright sunshine the chloroplasts in the lower layers of cells could get a sufficient amount of light energy for assimilatory activity. Consequently, assimilation had no reference to the surface area exposed by the leaves. In the shade, however, the surface exposed was the determining factor, since it is the superficially situated chloroplasts that determine the quantity of assimilation. Though it is ultimately the area of the assimilating surface (the aggregate 'reactive surface of the chloroplasts'[1]) exposed to light energy that determines dry-weight production in both cases, it will be erroneous to adopt always one standard of recording the dry weight, either by the unit-weight method or by the unit-area method, unless light energy is in excess—a conclusion which is confirmed by the results obtained on Cotton discussed below.

For these reasons, we have thought it best to compare the relative growth-rate with both the leaf area and the leaf weight during successive periods of growth in the different series, and to institute independent laboratory experiments to measure the assimilatory activity of the leaves per unit area and per unit weight under known environmental conditions at different stages of growth. It will then be possible to deduce the unit leaf-rate independently under field conditions, and this, when multiplied by the unit leaf area or the unit leaf weight in respective stages of growth, will enable one to determine to what extent assimilation controls growth and to

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[1] For an explanation of this term see Briggs, G. E. (8).

what extent it is controlled by growth (through, principally, variations in the quantity and the quality of the leaves with reference to assimilation). The data on the assimilatory capacity of the leaves per unit area and per unit weight at different stages of growth being yet incomplete, in this communication the relative growth-rate is compared with the area exposed by the leaves on the one hand and their dry weight on the other.

There is also the further question as to what units should be adopted for comparing leaf area and leaf weight with the relative growth-rate. If we assume that both leaf area and leaf weight are increasing at the same rate as the total dry weight, which is not very far from the truth till the flowering period is reached, the simplest way would be to compare variations in the relative growth-rate during successive periods of growth with variations in the leaf-area ratio and the leaf-weight ratio per unit dry weight of the plant during respective periods. The relative growth-rate is the average growth-rate during the periods of observations. It is therefore necessary to take the average leaf area and the average leaf weight of the plant per average unit dry weight during corresponding periods for comparison with the relative growth-rate. This is done by applying the formula suggested by the three authors for calculating the leaf-area ratio, viz. $\frac{L_1 + L_2}{W_1 + W_2}$, where L_1 and L_2 are the leaf area or the dry weight of the leaves, as the case may be, and W_1 and W_2 are the dry weights of the plant at the beginning and the end of the periods of observations respectively. The figures obtained in this way are given in Tables 5, 6, and 7, under the headings leaf-area ratio and leaf-weight ratio respectively for each series, and are graphically represented in Figs. 3, 4, and 5 for comparison with the relative growth-rate curves.

The growth-rate curve may be divided into three distinct phases in this connexion, viz.:

(*a*) The initial phase when the growth-rate agrees neither with the percentage leaf-weight ratio nor with the leaf-area ratio.

During this phase, the growth-rate lags behind the leaf-weight ratio or the leaf-area ratio. For instance, in the first series (Table 5) the leaf-weight ratio between the 9th and 14th day after germination is 53·5 as compared with 49·0 between the 9th and the 14th day. The growth-rates during the corresponding periods are, on the other hand, 60·16 per cent. and 81·92 per cent. This initial misfit between the growth-rate and the increase in the leaf substance may be due to the fact that, on an average, the leaves have not yet attained their maximum assimilating capacity in the early stage of growth.[1]

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[1] For subnormal photosynthetic activity during early stages of germination see Briggs, G. E. (9 and 10), and Irving, A. A. (11). The subnormality was noted, however, during very early stages of germination.

(*b*) The phase of active growth when the growth-rate curve runs parallel either to the leaf-weight ratio or to the leaf-area ratio, as the case may be, according to the season of growth.

In the first series, when plants were exposed to bright sunshine, the growth-rate curve runs parallel with the curve of leaf-weight ratio till the maximum increase in the growth-rate is reached. In the second series, however, when the bright weather continued only for a short period, the

TABLE 5. *Cotton.—First Series.*

(Germinated 14th of May 1923.)

Days after Germination.	*Relative Weekly Growth-rate Per cent.*	*Leaf-weight Ratio × 100.*	*Remarks.*
1	2	3	4
9			
	60·1	53·5	
14			
	81·9	49·0	
21			
	84·6	50·0	
28			Hot and stormy winds blowing during this period.
	0·34	50·0	
35			
	82·9	41·1	
42			
	82·4	50·3	
49			Rains commenced.
	117·02	51·7	
56			
	16·15	50·9	
63			
	20·25	49·9 [1]	
83			Flowering commenced.
	15·37	46·7 [1]	
97			
	1·02	33·3	
119			

growth-rate is hardly influenced by the leaf-weight ratio; and in the third series, when, with the prevalence of cloudy weather, light appears to be limiting the assimilatory activity of the leaves, there is no correlation between growth-rate and the leaf-weight ratio. On the other hand, the growth-rate curve in the active phase in the second and the third series follows closely the course of the leaf-area ratio. These results confirm Müller's observations referred to above. The question of subnormal photosynthetic activity does not appear to come in here in any way.

It is also interesting to note in this connexion that the area exposed by the leaves per unit weight of the leaf substance varies considerably with the season of growth. The area exposed by the leaves per gramme dry weight

[1] Reproductive organs are also included in these.

of the leaf substance in the second and the third series is graphically repre-
sented in Fig. 6 (compare also column 7 in Tables 2 and 3).

It will be noticed from an examination of the figure that the leaves in
the third series are, except in the initial stage, at any time thicker than those
in the second series which obtained a greater amount of light energy during
the growing period, a result quite contrary to expectation. Further, the
leaves grow gradually thicker in both the series from the time the *maximum*

TABLE 6.   *Cotton.—Second Series.*

(Germinated on the 6th of June 1923.)

Days after Ger-mination.	Relative Weekly Growth-rate Per cent.	Leaf-area Ratio.	Leaf-weight Ratio × 100	Remarks.
1	2	3	4	5
1				
8	6·7		...	Hot and stormy winds un-favourable to growth.
15	c·97	130·2	52·78	
22	52·86	135·64	48·34	Rains commenced.
29	133·6	186·44	56·36	
36	104·82	172·47	56·24	
43	88·88	150·43	61·75	Continuously heavy rains.
50	127·0	120·71	61·42	
57	19·34	116·13	61·18	
64	24·27	107·28	61·02	Flowers appeared on the plants.
71	10·69	91·86	55·91	
79	5·82	81·03	50·93	

increase in the growth-rate takes place. In the second series, where the
maximum increase takes place some time after germination, the leaves are
thicker in the beginning and grow thinner simultaneously with the *maximum*
increase. But in the third series, where the maximum increase in growth-
rate occurs only a short time after germination, the leaves attain their
maximum thinness in the beginning and begin to grow gradually thicker.
This later decrease in the area exposed by the leaves per unit dry weight of
the leaf substance might be one of the factors influencing the decrease in
the growth-rate during later phases of growth, especially if light energy is
not in sufficient quantity to reach the chloroplast granules in the lower
layers of cells in the leaf.

(*c*) The last phase of growth when again the growth-rate curve shows
agreement neither with the leaf-weight ratio nor with the leaf-area ratio.

COTTON
FIRST SERIES

FIG. 3

COTTON
SECOND SERIES
FIG. 4
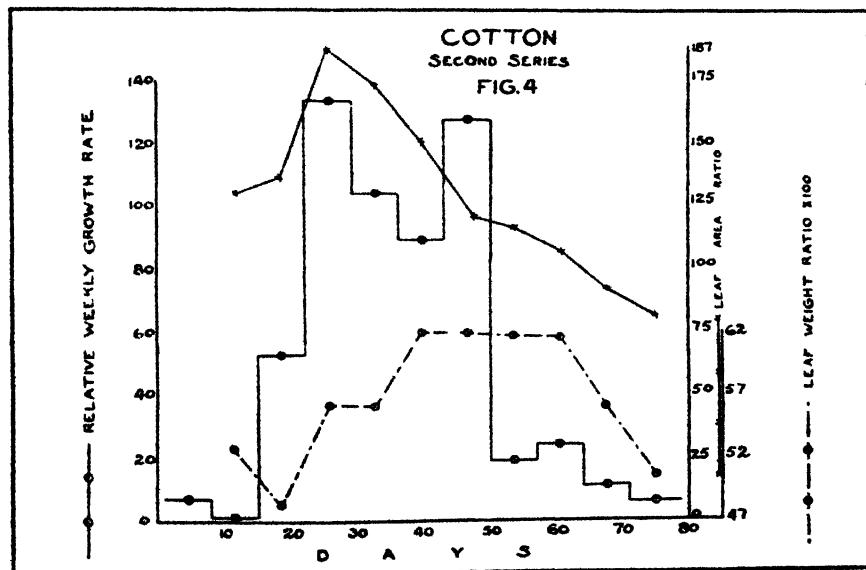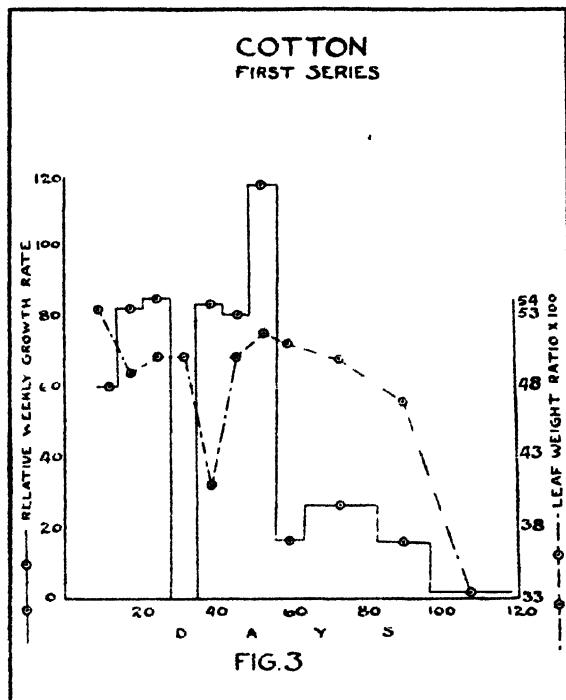
This phase occurs at about the flowering period in all the three series. During the vegetative period, new vegetative buds and young leaves are produced in abundance, but there is also a simultaneous shedding of the

TABLE 7.   *Cotton.—Third Series.*

(Germinated on the 15th of July 1923)

Days after Germination.	Relative Weekly Growth-rate. Per cent.	Leaf-area Ratio.	Leaf weight Ratio × 100.	Remarks.
1	2	3	4	5
1				
	28·2	...	· ·	
11				
	106·24	176·73	54·69	
18				
	80·44	145·15	54·64	
25				
	73·01	129·0	60·81	
32				
	30·15	121·94	58·71	
40				
	56·97	110·86	53·56	
47				Flower-bud appeared on one
	11·60	85·52	47·94	of the plants.
61				
	23·34	60·02	41·15	
75				Bolls appeared.

COTTON
THIRD SERIES
FIG. 5
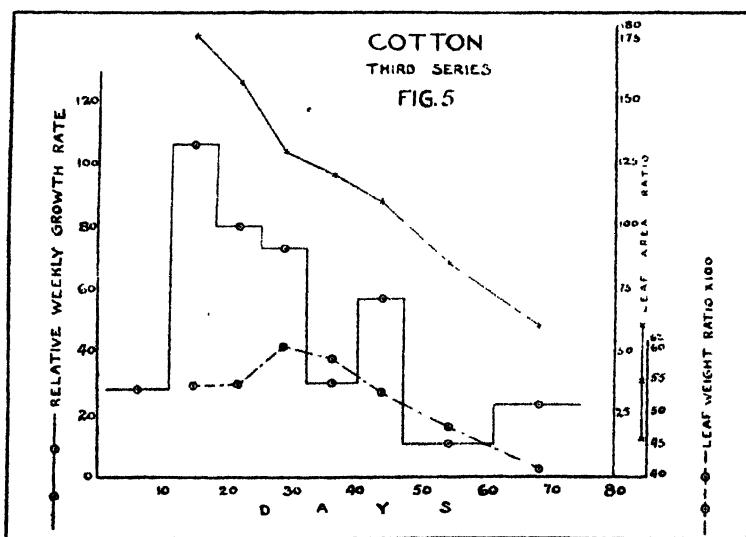
very old leaves, the average quality and quantity of the leaves remaining thus unaltered. During the flowering period, the flower-buds begin to replace the leaf-buds and the relative dry weight of the roots also increases

slightly (compare Tables 1, 2, and 3 respectively for each series). The increase in the dry weights of the reproductive organs and the roots is mainly at the expense of the leaves, the stem tissue showing only a small relative decrease. The leaves grow thicker also, and there is a consequent
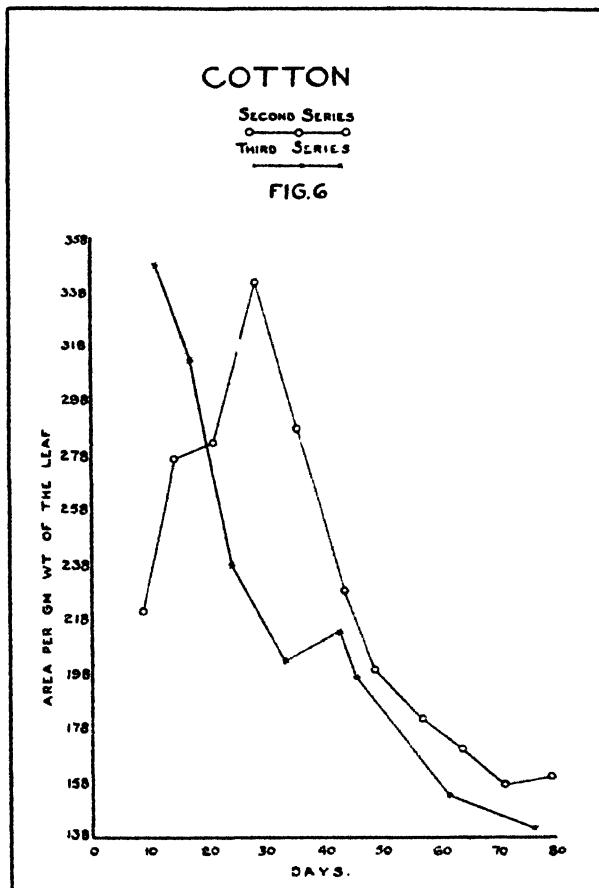
decrease in the area exposed. But the fall in the relative growth-rate in the last phase of growth is greater than can be expected by the decrease either in the relative dry weight of the leaves or the area exposed by them per unit dry weight of the plant. This must be due to the falling off in the assimilatory capacity of the leaves per unit dry weight and per unit area, since the average quality of the leaves with reference to assimilation is also altered, the proportion of the older leaves increasing gradually. Briggs[1]

[1] Compare (8), where he has discussed the question of subnormal photosynthetic activity of leaves due to old age.

has noted that the assimilatory capacity of even the young leaves falls off with increasing age of the plant. Briggs (8) is inclined to the view that the falling off is due to the change in the 'assimilating protoplasm' (the protoplasmic factor), and he explains that the subnormal photosynthetic activity in general is due to reduction in the ' reactive chloroplast surface ', which is not necessarily the same as the actual chloroplast surface. But with regard to the older leaves there is also the question of diffusion of CO_2 due to the immobility of the stomata combined with their partial or complete closure as the leaves advance in age. The relative values of these factors is a question which is yet to be investigated completely.

It may be seen from the foregoing description that the growth-rate of a plant as measured by the dry-weight method is controlled by either the leaf area or the leaf weight only for a short period of growth. On the other hand, the total capacity for dry-weight production by the plant is itself influenced according to different stages of growth by a number of internal factors which can be grouped together mainly under two headings, viz. (1) those that control the production and the development of the leaf buds as opposed to the flower buds, and (2) those that affect the assimilating capacity of the leaves per unit area and unit dry weight. These two sets of factors will vary according to the various external factors to which the plant is subjected and according to the internal ' periodicity '[1] of the protoplasm. The capacity for producing leaf buds differs in different plants, and, indeed, in different seasons of growth in one and the same plant. Thus, a cotton crop grown late in the rainy season comes to maturity sooner than the one grown earlier. Many external factors contribute to this result, the temperature and humidity being two of the most important.[2] The value of the internal factors alone in this connexion could be much more easily estimated if it were possible to keep all the external factors constant during the entire period of growth. This being a difficult task under field conditions, the only alternative is to keep a continuous record of humidity, light energy, and temperature, and to find out the percentages of correlation between the external factors on the one hand and the growth-rate and the leaf production on the other. It would then be possible to determine the value of the internal factors and to ascertain the quantitative effect of external factors on them.

For an elucidation of the factors that affect the capacity of the leaves for assimilation per unit area and unit dry weight one would require a much more thorough knowledge of the mechanism of assimilation than is available at present, and considerable amount of laboratory investigation is required in this direction. But a beginning under field conditions is made, as

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[1] The word ' periodicity ' is used in a very broad sense, meaning thereby any internal change due to change of season or period of growth.

[2] Compare Balls (12).

mentioned above, by measuring the assimilatory powers of the young and the old leaves per unit area and unit dry weight under known conditions of external factors during different stages of development on the same crop. One would then be in a position to compare the average assimilating power of the leaves with the dry-weight production by the plant at any stage of growth and to elucidate the factors that influence the capacity of the leaves with reference to assimilation.

C. Comparison between the relative growth-rate on the one hand and variations in the respiratory indices of the plant and its parts throughout the life-cycle on the other.

In *Helianthus*, Kidd, West, and Briggs (2) have noted that the respiratory index decreases with the increasing age of the plant. Their curve of respiratory activity at field temperature follows closely, however, the growth-rate curve, with an initial increase and a sudden decrease later. The results obtained on the Cotton crop (first series) are recorded in Table 8, and are drawn against time in Fig. 7.

TABLE 8. *Cotton.—First Series.*

(Germinated on the 14th of May 1923.)

Relative Growth-rate and Respiratory Indices.

Number of Days after Germination.	Weekly Relative Growth-rate of the Plant. Per cent.	Entire Plant.	Root.	Stem.	Leaves.	Remarks.
9		3·89	7·473	3·323	3·755	
	60·1					
14		3·23	4·28	2·64	3·32	
	81·9					
21		3·80	4·443	2·45	4·405	
	84·6					
28		3·31	3·665	2·250	3·565	Hot and stormy winds blowing during this period.
	0·34					
35		2·81	2·94	2·27	3·465	
	82·9					
42		2·46	4·33	2·31	3·89	
	80·4					
49		3·89	4·715	2·815	4·005	Rains commenced.
	117·02					
56		3·70	5·34	2·50	3·69	
	16·15					
63		3·43	3·99	1·75	3·525	Heavy rains commenced.
	26·25					
83		2·33	3·275	1·165	2·88	Flowering commenced.
	15·73					
97		1·78	2·00	0·975	2·3	
	1·79					
119		1·29	1·425	0·83	1·845	

Respiratory Indices.

X 2

COTTON

GERMINATED ON 14TH MAY.

1923

———○——— RESPIRATORY INDICES
OF LEAVES

———△——— RESPIRATORY INDICES
OF STEM

———○——— RESPIRATORY INDICES
OF ROOT

———○——— GROWTH RATE

----○---- RESPIRATORY

INDICES OF THE
WHOLE PLANT

NUMBER OF·DAYS AFTER GERMINATION

FIG.7
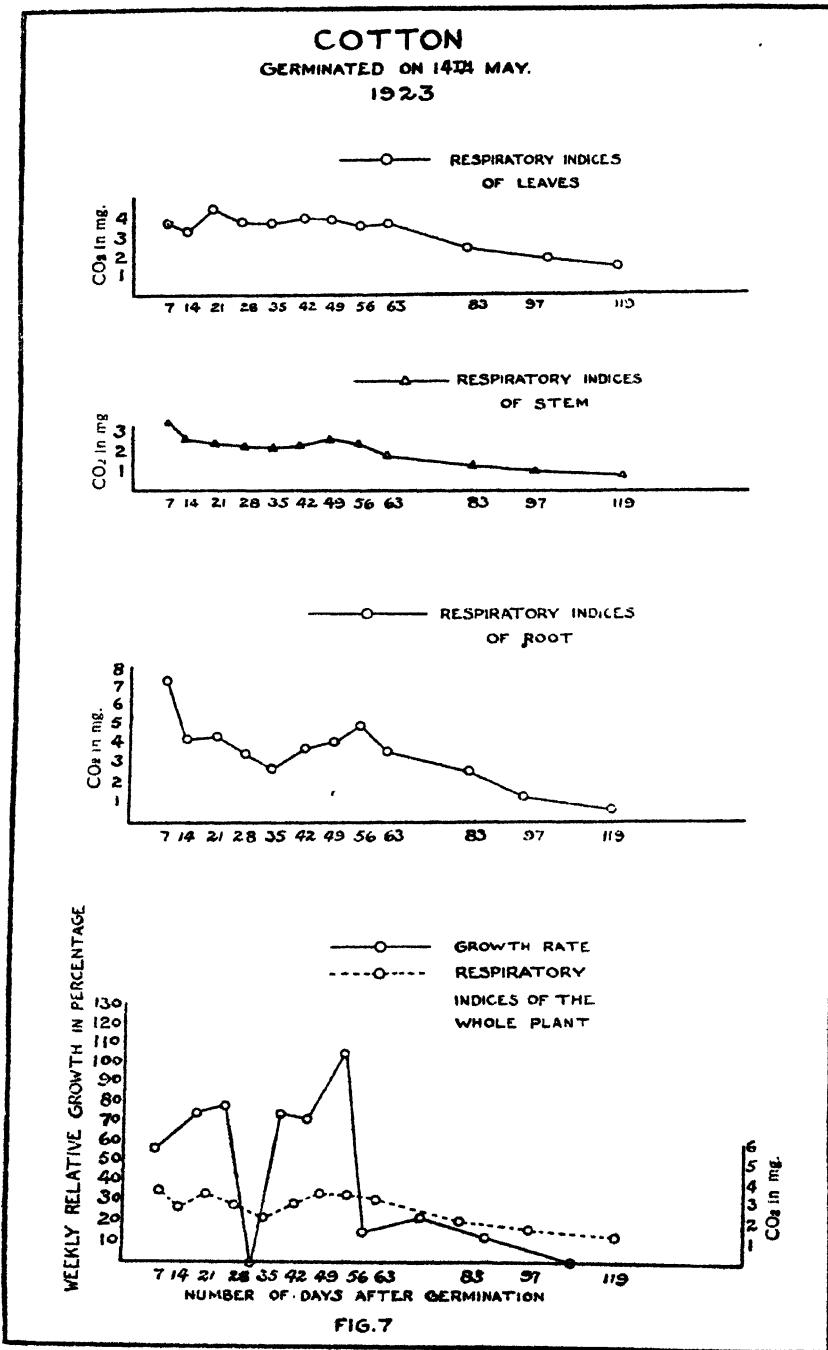
The respiratory index of the entire plant shows an initial high value which falls slightly later and remains practically constant with slight variations till the 63rd day, when the flowering period begins. After the flowering period there is a sudden decrease in the values. The same phenomenon is noticed in all the organs of the plant—the roots, the stem, and the leaves. But in the roots the initial value is much higher. It is doubtful, however, whether this high initial value in the roots represents the physiological truth, since it was noticed that the effect of time factor was practically nil on the respiration of the roots experimented upon on the 9th and the 14th days, respectively. This must be due to the fact that the roots, during the earlier stages of growth, contain a larger store of respirable material than the stem and the leaves.

The effect of time factor is similar in all the organs later and the values are thus comparable. On a comparison of the respiratory indices in the three parts separately it will be noticed that the roots, except during the last two periods, respire most, then the leaves, and lastly the stem. It cannot be said with any degree of certainty without accurate measurements whether this difference in the respiratory indices of the roots, stem, and the leaves is partly or wholly due to the different proportions of non-living tissues to the living tissues, and the consequent difference in the quantities of the respiring cell-matter. At any rate, part of this difference must be due to the differential supply of respirable material in the three organs without any reference to the quantity of the respiring cell-matter.

We now come to the decreasing stage of the respiratory index. The fall sets in sooner and is greatest in the stem tissue in comparison with the roots and the leaves. The fall in all the organs must partly be due, as Kidd, West, and Briggs state, to the increasing proportion with increasing age of non-living tissues per unit dry weight, the more so in the stem tissues than in the root—or the leaf—tissues. One must agree with them also that the entire fall cannot be accounted for in this way, and that the true cause must be sought for in some internal factor or factors.

In this connexion, the results obtained in *Helianthus* on the respiratory index of the stem apices are interesting. As the three authors state, we are dealing here with a tissue which is mainly meristematic and might therefore be expected to retain its original respiratory activity. But in their experiments the respiratory index of the stem apices did not remain constant, but decreased rapidly at first from the 22nd day to the 53rd day after germination, after which the fall was negligible till the 64th day. It must be noted, however, that the flower rudiments had already begun to appear in the 'top cluster' on the 53rd day, and after the 64th day it consisted entirely of the inflorescence. It is also not clear from their statements what number of young leaves were included in the 'top cluster' which they have experimented upon. If the top cluster contains a varying number of young

TABLE 9. *Cotton.—First Series.*

Comparison of the Respiratory Index of the Meristematic Tissue with that of the General Plant and its Parts.

Meristematic Tissue.		General Plant and its Parts.									
			General Plant.		Roots.		Stem.		Leaves.	
Date.	Respiratory Index.	Date.	Respiratory Index	Percentage to Meristematic Tissue.	Respiratory Index.	Percentage to Meristematic Tissue.	Respiratory Index.	Percentage to Meristematic Tissue.	Respiratory Index.	Percentage to Meristematic Tissue.
4th Aug. 82nd day after germination.	5·086	5th Aug. 83rd day after germination.	2·33	45·81	3·75	64·39	1·165	22·90	2·88	56·6
2nd Sept. 111th day after germination.	5·05	10th Sept. 119th day after germination.	1·29	25·54	1·425	28·21	0·83	16·43	1·845	36·53

leaves the proportion of comparatively differentiated leaves, and consequently the quantity of the respiring cell-matter, will differ, and that might partly be the cause of variation in the respiratory index.

Experiments in this direction were also conducted in this laboratory, taking always two folded leaves in addition to the closed apical buds which contained no flower rudiments. As this point was taken up rather late in the season only two results are available for comparison. A reference to Table 9 will show that the respiratory index of the meristematic tissue is higher than that of all the parts observed at about the same period of growth, and remains constant from the 82nd day to the 111th day, even though there was a rapid fall in the respiratory index of the entire plant during this period. Further observations are necessary before coming to a definite conclusion on this point, especially in view of the contradictory results obtained in *Helianthus* and Cotton.

Finally, on a comparison of the respiratory index with the growth-rate, it will be seen that the former follows the latter closely except in the very early phase of growth. In the first series of Cotton where there is a continued constant phase of growth before the maximum is reached, the respiratory index follows a parallel course before it begins to fall; while in the case of Balsam, where there is no continued constant phase of growth, the index falls simultaneously with the growth-rate, as can be seen from Table 10 and from Fig. 8 on p. 304.

TABLE 10. *Balsam.*

(Germinated on the 10th of June 1923.)

Relative Growth-rate and Respiratory Indices.

Number of Days after Germination.	Weekly Relative Growth-rate of the Plant. Per cent.	Respiratory Indices.				Remarks.
		Entire Plant.	Root.	Stem.	Leaves.	
7		4·235	...	...	...	Plants too small to be divided into the three parts.
	25·65					
28		7·67	12·3	5·33	6·135	
	156·1					
37		6·80	9·65	4·79	6·45	
	84·28					
51		5·25	6·90	3·60	5·2	Flowering commenced on the 40th day.
	33·6					
63		3·63	4·28	2·55	3·79	
	13·51					
85		1·78	2·03	1·17	2·35	

The same general interpretation can be put on the figures obtained by Kidd, West, and Briggs on *Helianthus*. The later decrease in the respiratory index may or may not be correlated with the nuclein nitrogen content
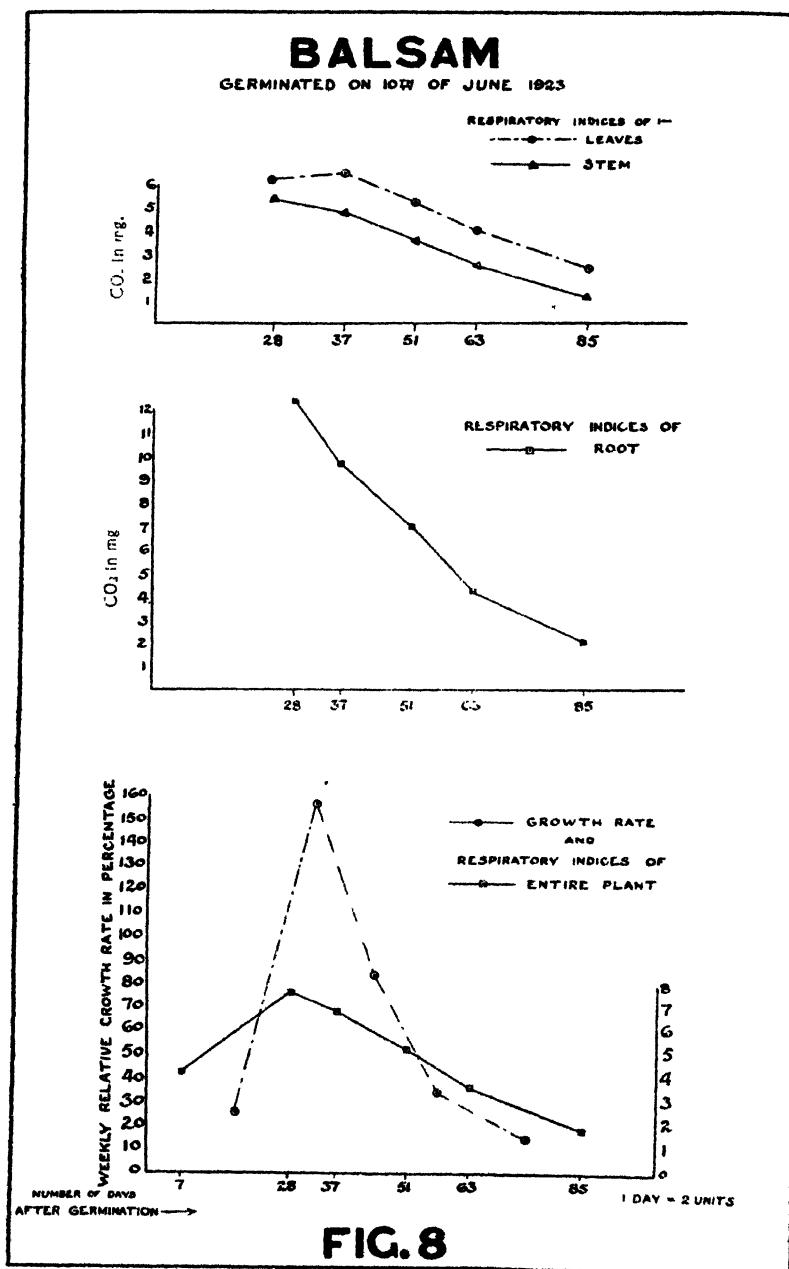
FIG. 8

of the plant, drawn attention to by the three authors from results obtained by Palladin (18). But their suggested relation between growth-rate and respiratory index on the one hand and protein nitrogen content on the other can be upheld in one respect, viz. that both these physiological processes are the result of one and the same set of causes. The course of the respiratory index expresses merely the intensity of the series of 'protoplasmic activities' which influence growth-rate, though part of this correlation must be traced to the relative supply of respirable material in different plants with different capacities for producing dry weights. Looked at from this point of view, respiration seems to have little or nothing to do with the increase or decrease in the growth-rate. An explanation of the varying growth-rates of the plants is therefore to be sought for in the internal factors mentioned above, viz. those which influence the quantity and the capacity of the assimilating surface.

IV. SUMMARY.

1. The growth of the Cotton plant is measured by the dry-weight method, and the relative growth-rate per week is calculated on an exponential basis.

2. The relative growth-rate curves of plants grown in different periods of the year are compared with each other. The growth-rate curves show a *maximum* increase which is reached *sooner* or *later* according to the duration of the vegetative period, and begin to fall off later. The shorter the vegetative period, the earlier is the *maximum* reached. The *maximum* increase supplies also the necessary stimulus to the plant to set itself to produce reproductive organs, with an intervening latent period of about one month. The maximum increase is thus a phenomenon of some physiological importance and requires further elucidation. The results obtained do not lend any support to the existence of a 'grand period of growth' with a continued maximum rate of growth.

3. The growth-rate curves are also compared with variations in the leaf-weight ratio and the leaf-area ratio. The growth-rate curve is divided into three phases in this connexion:

(*a*) An initial phase when the growth-rate curve agrees neither with the leaf-weight ratio nor the leaf-area ratio. It is suggested that this might be due to the fact that the leaves, on an average, have not yet attained their maximum assimilating capacity.

(*b*) An active phase of growth when the relative growth-rate curve runs parallel to the curve of either the leaf-weight ratio or the leaf-area ratio. In the plants grown in bright weather during the summer, the growth-rate is influenced by the percentage weight of the leaves, while in the cloudy weather

it is the area exposed that appears to be the determining factor. The area exposed per unit dry weight of the leaves also changes as growth advances. In all the series observed the leaves grow gradually thicker after the maximum in the growth-rate has taken place.

(*c*) The last phase of growth, when there is greater decrease in the growth-rate than can be accounted for by the decrease either in the percentage leaf-weight ratio or the leaf-area ratio. It is suggested that this is due to the falling off in the assimilating capacity of the leaves during the later stages of growth, partly because the condition of the stomata retards diffusion and partly because of other internal factors of which the 'protoplasmic factor' of Briggs might be the one mainly concerned.

. 4. A general discussion of the internal and the external factors concerned in the dry-weight production by the plant is included.

5. The growth-rate curve is compared with the course of the respiratory index in the plant and its parts throughout the entire period of growth. The course of the respiratory index is found to run parallel to the growth-rate curve, a conclusion which can be extended to the results of Kidd, West, and Briggs on *Helianthus*. The results on the respiratory values of the meristematic tissues differ, however, from those of the three authors. It is concluded that the course of the respiratory index merely expresses the intensity of the series of 'protoplasmic activities' which influence growth-rate. It does not seem to have any influence on the decreasing or the increasing rate or growth.

APPENDIX.

I. Cotton.—First Series.

(Germinated on the 14th of May, 1923.)

A. Dry Weights during successive Periods of Growth.

Date.	Number of Days after Germination.	Number of Plants taken for Observation.	Fresh Weight per Plant.				Dry Weight per Plant.				Remarks.
			Total.	Leaves.	Stem.	Roots.	Total.	Leaves.	Stem.	Roots.	
			grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	
22.5.23	9	23	0·97	0·497	0·325	0·148	0·0717	0·0427	0·0227	0·0063	
27.5.23	14	15	1·2225	0·585	0·362	0·332	0·1101	0·0547	0·0383	0·0171	
3.6.23	21	13	2·445	1·119	0·729	0·597	0·250	0·123	0·084	0·043	
10.6.23	28	5	4·278	1·881	1·134	1·263	0·5831	0·2941	0·095	0·094	Hot and stormy winds.
17.6.23	35	5	4·976	2·366	1·35	1·26	0·583	0·257	0·195	0·101	
24.6.23	42	3	9·831	4·553	2·873	2·405	1·33	0·60	0·52	0·21	
1.7.23	49	2	23·694	12·374	6·00	5·307	2·979	1·57	0·916	0·493	Heavy initial showers.
8.7.23	56	1	64·874	29·23	13·03	22·614	9·624	4·954	2·551	2·119	Continuously heavy rains commenced.
15.7.23	63	1	73·591	33·88	16·195	23·516	11·305	5·708	3·501	2·096	
4.8.23	83	1	Not taken.		1·57	5·96	23·933	11·897[1]	5·826	6·21	Flowering commenced.
18.8.23	97	1	Not taken.		31·5	45·2	32·534	14·481[1]	11·251	·802	
9.9.23	119	1	94·5	26·6	27·1	40·5	33·609	7·478	10·631	7·6 Fruits 7·9	

[1] Include also the weight of the reproductive structures.

II. Cotton.—Second Series.

(Germinated on the 6th of June 1923.)

Dry Weights and Leaf Area during successive Periods of Growth.

Date.	Number of Plants taken for Observation.	Fresh Weight per Plant.	Dry Weight per Plant.				Area exposed by Leaves per Plant.	Remarks.
			Total.	Roots.	Stem.	Leaves.		
		grm.	grm.	grm.	grm.	grm.	sq. cm.	
6. 6. 23	110	...	0·0476	...	...	...	...	Plants too small to be divided into separate parts— root, stem, and leaves.
13. 6. 23	5	0·4951	0·0509	0·0028	0·0187	0·0294	6·52	Hot and stormy winds unfavourable to growth.
20. 6. 23	5	0·552	0·0514	0·005	0·0218	0·0246	6·8	
27. 6. 23	5	0·903	0·0872	0·0104	0·0344	0·0424	12	
4. 7. 23	2	2·6107	0·3317	0·04	0·098	0·1937	66·1	Rains commenced. Heavy initial showers.
11. 7. 23	2	7·911	0·9462	0·186	0·2252	0·535	154·3	
18. 7. 23	2	16·4305	2·3015	0·3585	0·4725	1·4705	334·25	Continuously heavy rains commenced.
25. 7. 23	2	52·317	8·195	1·033	2·1855	4·977	999·7	
1. 8. 23	2	55·0045	9·953	1·57	2·256	6·127	1107·9	
8. 8. 23	2	61·6025	12·6875	1·95	3·5625[1]	7·165	1228·95	Flower bud appeared on one of the plants.
15. 8. 23	2	62·555	14·1108	2·472	3·829[1]	7·818	1232·65	
22. 8. 23	2	65·592	14·9645	2·882	5·09[1]	6·9925	1123·5	

III. Cotton.—Third Series.

(Germinated on the 15th of July 1923.)

Dry Weights and Leaf Area during successive Periods of Growth.

Date.	Number of Plants.	Fresh Weight per Plant	Dry Weight per Plant.				Area exposed by Leaves per Plant.	Remarks.
			Total.	Roots.	Stem.	Leaves.		
		grm.	grm.	grm.	grm	grm.	sq. cm.	
15. 7. 23	25	...	0·124	...	...	...	...	
25. 7. 23	20	2·275	0·185	0·0186	0·0552	0·1116	38·9	
1. 8. 23	15	5·8284	0·538	0·0995	0·154	0·2838	88·88	
8. 8. 23	5	8·0984	1·202	0·193	0·325	0·684	163·68	
15. 8. 23	5	16·41	2·494	0·394	0·5372	1·5634	313·12	
23. 8. 23	4	22·225	3·521	0·736	0·816	1·9685	420·4	
30. 8. 23	4	32·6925	6·2215	1·038	1·934	3·25	659·7	Flower bud appeared on one of the plants.
13. 9. 23	2	31·18	7·851	1·25	3·1535[1]	3·4475	535·25	
27. 9. 23	2	52·075	12·5225	3·0575	4·5275[1]	4·9375	687·55	Bolls appeared.

[1] Include also the weight of the reproductive structures.

Cotton.—Series I.

Respiration : mg. CO_2 per hour per grm. dry weight through successive two-hour periods.

(The first period is preceded by an initial preliminary period of two hours.)

Number of Days after Germination.	Parts of Plant.	Period I.	Period II.	Period III.	Number of Days after Germination.	Parts of Plant.	Period I.	Period II.	Period III.
9	Leaves	4·36	3·79	3·10	49	Leaves	5·75	4·75	4·09
	Stem	4·21	3·09	2·66		Stem	3·29	2·56	1·98
	Root	7·67	7·4	7·35		Root	6·1	4·28	3·77
14	Leaves	3·92	3·28	2·25	56	Leaves	4·00	3·26	3·33
	Stem	3·3	2·6	2·02		Stem	3·00	2·32	2·16
	Root	4·26	4·22	4·35		Root	6·7	4·95	4·38
21	Leaves	5·65	4·30	3·31	63	Leaves	3·99	3·4	3·18
	Stem	3·04	2·41	1·89		Stem	2·05	1·69	1·52
	Root	5·64	4·40	3·28		Root	5·11	3·7	3·05
28	Leaves	4·7	3·85	3·50	83	Leaves	2·75	2·32	2·08
	Stem	2·94	2·21	1·59		Stem	1·36	1·13	1·00
	Root	4·14	3·43	2·92		Root	3·72	3·23	2·88
35	Leaves	4·26	3·46	2·67	97	Leaves	2·62	2·34	1·95
	Stem	2·68	2·26	1·88		Stem	1·09	0·97	0·87
	Root	—	3·01	2·86		Root	2·2	2·02	1·79
42	Leaves	4·95	3·53	3·03	119	Leaves	2·01	1·80	1·72
	Stem	2·83	2·39	1·95		Stem	0·91	0·83	0·75
	Root	5·28	4·03	3·65		Room	1·50	1·40	1·37

Balsam.

(Germinated on the 10th of June 1923.)

Date.	Number of Days after Germination.	Number of Plants taken for Observation.	Fresh Weight per Plant.				Dry Weight per Plant.				Remarks.
			Total.	Leaves.	Stem.	Roots.	Total.	Leaves.	Stem.	Roots.	
			grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	
17.6.23	7	43	0·125	—	—	—	0·0083	—	—	—	Plants too small to be divided into root, stem, and leaves.
8.7.23	28	20	1·9419	0·8477	0·3535	0·7407	0·0894	0·0511	0·0143	0·024	
17.7.23	37	4	12·715	5·8	3·015	3·9	0·6697	0·4002	0·131	0·1385	
31.7.23	51	3	41·74	7·451	17·039	17·25	3·633	1·812	0·879	0·743	Flowering began on the 40th day.
12.8.23	63	2	58·99	9·356	38·065	17·565	6·484	2·8785[1]	1·9055	1·7	
3.9.23	85	1	88·37	13·04	49·17	26·16	10·932	3·764[1] 0·512[2]	4·476	2·18	

Respiration : The results are given in terms of mg. CO_2 per hour per grm. dry wt. Periods I, II, and III each consisted of two hours; they were preceded by a preliminary period of two hours.

Expt. No.	Plant Organs.	Period I.	Period II.	Period III.	Expt. No.	Plant Organs.	Period I.	Period II.	Period III.
I	{ Plants too small to be divided.	4·14	4·10	4·18	IV	{ Leaves	6·05	5·35	4·2
						Stem	4·1	3·45	3·2
						Root	7·4	6·9	6·4
II	{ Leaves	6·15	5·90	6·35	V	{ Leaves	4·15	4·01	3·75
	Stem	4·51	4·88	6·59		Stem	2·62	2·54	2·48
	Root	11·45	11·25	14·24		Root	4·60	4·28	3·96
III	{ Leaves	7·18	6·35	5·84	VI	{ Leaves	2·5	2·3	2·25
	Stem	4·95	4·75	4·67		Stem	1·17	1·17	1·17
	Root	8·61	9·9	10·45		Root	2·1	2·01	2·0

[1] The dry weight of reproductive organs included.
[2] Reproductive organs only.

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Notes on the Cones of the Calamostachys Type in the Renault and Roche Collections.

BY

ISABEL M. P. BROWNE.

With Plates IX–XI and three Figures in the Text.

OWING to the kindness of the authorities at the Muséum d'Histoire Naturelle at Paris I was able during recent visits to that city to study the sections of Calamarian cones in the Renault and Roche collections. M. Costantin, Director of the Museum, was most kind in facilitating in every way my work on these sections. I also received much help and encouragement from M. Fritel, another member of the staff. In particular, I am indebted to the authorities of the Museum in Paris for permission to have certain of the preparations photographed by M. Cintract and for allowing me to publish these photographs. M. l'Abbé J. Chevalier and M. L. Gillot, respectively President and Secretary of the Société d'Histoire Naturelle d'Autun, most kindly allowed me to study such of Renault's sections as are in the possession of the said society at Autun. To all these I wish to offer my sincere thanks; it is entirely owing to their hospitality and indulgence that it has been possible to collect the information now published. I am also indebted to Dr. Scott for most helpful criticism.

PART I. AN UNNAMED CONE OF A *CALAMODENDRON* FROM GRAND'CROIX (*CALAMOSTACHYS MAGNAE-CRUCIS*, N.SP.)

Among the fructifications ascribed by Renault to the genus *Calamo-dendron* was a cone of which he published only two short notices (Renault, 14, p. 252; 15, p. 453). He never figured this fossil nor gave it a specific name. As is well known, Renault held to the end of his career to

the dogma, first enunciated by Brongniart, that the Calamariae included Cryptogamic forms devoid of, and Phanerogamic forms provided with, secondary xylem. He regarded the cone in question as belonging to the latter group, which included the genus *Calamodendron*, and referred to it as the male fructification of a *Calamodendron* from Grand'Croix, near the Rive de Giers.

It will be seen from the following summary of Renault's descriptions that the cone was one that would nowadays be included in the genus *Calamostachys*.

The axis bore at intervals of about 4 mm. whorls of twenty-four sterile bracts, and contained eight main bundles, provided with well-marked lacunae. Outside and between the main bundles were sixteen smaller strands, with or without lacunae, which are said to be ' in relation with ' the sixteen sporangiophores that were inserted in the middle of each internode. The sporangiophores were peltate structures; each bore four sporangia. The latter were about 1·6 mm. in height, of an average width of 0·5 mm. and about 2·1 mm. in radial depth. The thickenings of the cells of the sporangial walls fitted into one another in a complicated manner. The spores (Renault's pollen grains) were very small, and were found still grouped in tetrads, surrounded by the cuticularized wall of the spore-mother-cell. Each spore possesses two envelopes, and is said to be pluricellular.

The fructification thus described presents a combination of characters unknown among petrified cones, and it will be convenient to be able to refer to it by name. As in both Renault's notices of it it is referred to as the cone of a *Calamodendron* from Grand'Croix, I propose to call it *Calamostachys magnae-crucis*.

In the Renault collection is a transverse section of a cone, about 10 mm. in diameter, which clearly belongs to *C. magnae-crucis*. This section (Pl. IX, Fig. 1) passes very near to the level of insertion of the sporangiophores, since it shows the bases, slightly more prominent on one side, of sixteen of these organs. The section traverses portions of thirty-two closely approximated sporangia, rather more than 2 mm. in radial depth, the cells of the walls of which are thickened in a complicated manner. Each spore is surrounded by two envelopes, and the tetrads have very much the appearance of those figured by Renault as representing the ' pluricellular pollen grains ' of his *Calamodendrostachys Zeilleri* (Renault, 16, Pl. LX, Fig. 8). As early as 1894, however, doubt was thrown on the pluricellular nature of the spores in the French Calamariae described by Renault. Drs. Williamson and Scott held that the pluricellular appearance of such spores was probably illusory and due to contraction of the endosporium (23, p. 912). The small size of the spores of *C. magnae-crucis*, which, like those of Renault's *Calamodendrostachys*

Zeilleri, are about 45 μ in diameter, makes it very unlikely that they would be pluricellular. Moreover, though the two preparations of *C. magnae-crucis* described in the present paper include a very large number of spores, in no case does the section expose any of the so-called cells ; these are always seen through the more or less transparent coat of the spore, a fact that supports the view that Renault's supposed cell-walls represent folds of the endosporium, probably due to contraction during fossilization. Over a little less than half the circumference of the circle formed by the sporangia are eleven bracts cut more or less transversely.[1] The axis is about 2·2 mm. in diameter and contains eight large lacunae representing the main bundles, disposed rather regularly, one between the bases of every two sporangio-phores. In addition there appear to be sixteen small strands, or patches of darker tissue, lying externally to the main bundles, either in the bases of the sporangiophores or opposite to them. That these small patches of darker tissue may be regarded as vascular strands is shown by the presence in two of them of very small but distinct lacunae, presumably representing proto-xylem that has perished. At the same time the position of some of them within the bases of the sporangiophores suggests that they are traces and not true axial bundles.

The conclusion that this section was one of those on which Renault based his description of the cone of a *Calamodendron* from Grand'Croix is confirmed by the fact that the slide is labelled in his writing : ' 8 lacunes, 16 sporangiophores, 32.   , 24 feuilles, parois cellulaires des sporanges, pollen pluricellul.'[2]

The proportion of three bracts to two sporangiophores may prove to be sometimes only approximate (cf. p. 325). but it is an unusual one, characteristic, so far as we know, only of *C. magnae-crucis* among petrified Calamarian cones. There is, however, in the Roche collection at the Muséum d'Histoire Naturelle at Paris, a section, Section 2 of the catalogue, which seems to show the same proportion of bracts to sporangiophores and agrees very closely in structure with the section just described. This similarity seems to have been already recognized, for Roche's section is labelled ' *Calamodendron.* Pollen pluricellulaire.' It may safely be attri-buted to *C. magnae-crucis.*

Roche's specimen (Pl. IX, Fig. 2) is somewhat smaller than Renault's. The complete cone measures about 8 mm. across and the diameter of the axis is approximately 1·75 mm. There were but fourteen sporangiophores. The bracts, where portions of them are preserved, seem to be present in the proportion of about three to every two sporangiophores, which would suggest a total of twenty-one. The axis contained seven large lacunae,

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[1] Pl. IX, Fig. 1 does not show all of these.
[2] The number 32, followed by a blank, refers to the number of sporangia in the section. In the latter only two of the four sporangia borne by each sporangiophore are, of course, to be seen.

clearly corresponding to the eight large lacunae of the main bundles of Renault's specimen, and lying, as did these, between the bases of every two sporangiophores. There is also a somewhat smaller main lacuna, which is apparently dying out. Considerable portions of the stalks of three sporangiophores may be seen radiating outwards. On this side of the section small vascular strands are visible in the bases of the sporangiophores, though on the other side there is no sign of the smaller strands. The sporangia and spores appear to be in every way closely similar to the corresponding structures in Renault's specimen. Thus Roche's specimen confirms the impression left on the mind by Renault's that the small bundles are in reality the traces of sporangiophores. Renault's section, in which the traces on one side of the specimen are still in the axis, therefore presumably passes through the lower pairs of sporangia of the sporangiophores. In Roche's specimen the traces have already passed out, so that not only do the sporangia present belong to the upper series, but where the stalks of the sporangiophores pass out of the section the plane of the latter lies above them. Further, it is noticeable that on this higher side of the section the sporangia, where preserved, are contiguous in pairs, each pair being slightly separated from the neighbouring pairs. If the median lines of the stalks of the sporangiophores be followed outwards, it will be seen that on this side of the section the approximated sporangia belong to different sporangiophores.

Two more points concerning the traces of the sporangiophores remain to be emphasized. It will be seen, in the first place, that in both sections all the traces which can be distinguished seem to enter the sporangiophores on the sides of these organs remote from the nearest axial bundle. In the absence of any other axial vascular strands each of these bundles must obviously have given rise to two traces, and the fact that these traces enter the sporangiophores on the sides remote from the nearest axial bundles suggests that the latter alternated from one internode to the next, and that they gave off traces to the sporangiophores before undergoing anastomosis. If this was so the traces probably originated at or very near the level of the node below, as Renault says they did in *Calamostachys* (his *Calamodendrostachys*) *Zeilleri* (Renault, 16, p. 130), a cone which, as will be shown later, resembles in many points *C. magnae-crucis*. The sporangiophores of successive whorls were presumably superposed, for this seems to have been the rule in the great majority of Calamarian cones, but the alternation of the bundles may well have been accompanied by an alternation of the bracts of successive whorls. Such a combination of whorls of alternating bracts with intervening whorls of sporangiophores superposed to one another occurs in *C. Binneyana*, Carr., and *C. Ludwigi*, Weiss, and probably also in other species. In *C. Binneyana* the axial bundles remained unbranched, and the adjustment of the vascular system to the supplying of traces to

alternating bracts was effected by the torsion in the cortex of such traces as did not lie opposite to the bracts to which they were destined (7, p. 10). In *C. magnae-crucis* the adjustment of the vascular system to a similar combination of superposed sporangiophores and alternating bracts seems to have been effected by the alternation of the bundles and by the divergent course of the traces of the sporangiophores as they pass upwards and outwards through the cortex. Although the steep upward course of the traces allowed of their very gradual divergence, yet the actual extent of this divergence seems to have been as small as possible, so that the traces entered the sporangiophores where the latter were nearest to their points of origin. Unfortunately no transverse section is known passing through the bractigerous node, such as would give direct evidence of the origin of the traces of the sporangiophores. The circular outline of the small canals of the traces while in the axis and the absence of any indication of the emission of traces from the main bundles, especially from those in Renault's section, which passes just below the stalks of the sporangiophores, however, strongly suggest that the traces of the latter organs originated at the node below and passed steeply upwards. Text-fig. 1 shows in diagrammatic form the suggested relations of the vascular strands. It is not, of course, contended that such a regular system prevailed throughout the cones of *C. magnae-crucis*. On the

TEXT-FIG. 1. Diagram of the suggested course of the vascular strands in the axis of *Calamostachys magnae-crucis*, n. sp. *m.p.*, main bundles alternating from one node to the next ; *s.b.*, sporangiophore bundle; *i.s.*, insertion of sporangiophore; *s.t.*, trace of sporangiophore ; *o.s.t.*, origin of the sporangiophore trace ; *b.t.*, bract-trace.

contrary, there were clearly variations in the number of appendages in the whorls, since in the only two transverse sections identified the fertile whorls consisted respectively of sixteen and fourteen, and the sterile ones probably of twenty-four and twenty-one members. The diagram merely represents what was possibly the normal type of vascular system evolved, when there was no irregularity arising from fluctuations in the number of the appendages, as a response to or in correlation to the possession of superposed sporangiophores and alternating bracts.

The second point to be noted about the traces of the sporangiophores is that just after their entry into these organs they appear to be curved, almost crescentic, in form. This appearance is probably due to the fact that the trace, besides curving to one side so as to occupy the normal median position of traces in sporangiophores, is passing out of the (horizontal) plane of the section, so that just before it disappears a few only of its elements are visible.

It seems not unlikely that Renault never possessed a transverse section from the neighbourhood of the bractigerous node, for although he usually mentions the origin of the bract traces of his petrified Calamarian cones, he gives no information as to this point for the species under consideration. He merely says that some of the twenty-four bracts were inserted opposite to the main bundles and others opposite to the smaller bundles (our traces), a fact which could be deduced from the distribution of the bracts in the section shown in Pl. IX, Fig. 1. If, as seems to have been the case in both transverse sections, the proportion of bracts to sporangiophores was as three to two, and the axial bundles were half as numerous as the latter, then each bundle presumably gave off traces to three bracts. There is nothing surprising in this, for Dr. Hickling has shown that in *C. Binneyana* each bundle gave off traces to three or four bracts. In this species, it is true, the bundles appear often to have been double (Hickling, 7, pp. 7-10).

Renault says of his unnamed cone from Grand'Croix that the bracts turned upwards at a distance of about 4 mm. from the axis and that they attained a length of about a centimetre. As the internodes were 4 mm. in length the bracts shown in Pl. IX, Fig. 1 must have been cut about 6 mm. from their bases. At this level their greatest radial depth is in their median region, where they are about 1 mm. in radial depth. Each bract is prolonged on both sides into a thin, straight wing, and, including their wings, the bracts are about 1½ mm. in width. In the median region, but near to the adaxial surface, is an elliptical hypodermal strand of fibres, about ½ mm. in width. Outside this strand the upper (adaxial) surface of the bract is very slightly convex. In some cases the remains of the vascular bundle can be seen lining the abaxial (morphologically) lower side of the fibrous strand and forming, with the latter, a bluntly triangular patch. The bracts possessed a hypodermal layer of radially elongated cells, and this seems to have been in by far the greater number of cases the most externally situated layer preserved. The bracts are so closely approximated that their wings overlap. Usually one wing of each bract lies inside and the other outside the similar wings of neighbouring bracts; but in a few cases both wings of a bract lie inside or outside those of its neighbours.

Among the preparations of the Renault collection is a series of longitudinal sections through a cone which shows striking similarities to *C. magnae-crucis*, though, as will be seen later, the attribution of these sections raises

a problem of some complexity. These sections are all tangential, and may well have been cut at different depths from the same cone. In any case they all four obviously belong to the same species. The most extensive of these sections, of which Fig. 3 of Pl. IX is a photograph, is about $2\frac{1}{2}$ cm. long and at its widest point about 8 mm. in width. It includes portions of seven fertile and seven sterile whorls, the internodes being about 4 mm. long. The stalks of the sporangiophores are cut transversely and have the appearance of circular dots. Not all the stalks are preserved *in situ*, but it is usually possible to estimate their position, for where both stalks and sporangia are preserved it can be seen that the lower pair of sporangia (with reference to the organic apex of the cone) of a sporangiophore appear in the photograph (Pl. IX, Fig. 3) to converge pyramidally to a point just below the stalk of the latter organ. Consequently, the outline formed by the lower series of sporangia of a whorl is a zigzag, of which the prominences lie above the points of junction of the lower sporangia of a sporangiophore, while the depressions divide the lower sporangia of one sporangiophore from the lower ones of another. The upper series of sporangia fit into these depressions. Thus the sporangia of both series appear to be approximated in pairs ; but in the lower series the sporangia constituting a pair belong to the same, while in the upper series they belong to different sporangiophores. The appearance of the sporangia, the thickening of the cells of the sporangial walls, the appearance of the very small spores still connected in tetrads and surrounded by the membrane of the spore mother-cell, the folds of the endosporium, giving the spores a fallacious appearance of being pluricellular, all vividly recall the corresponding features in *C. magnae-crucis.*

From a study of the section shown in Pl. IX, Fig. 3, five conclusions as to the structure of the cone can be deduced. Firstly, in the middle of the portion preserved the sporangiophores of successive whorls are distinctly superposed to one another, while those of the uppermost whorl and those of the second whorl from the lower end seem to alternate somewhat irregularly with those of the whorls immediately below. This apparent irregular alternation of the sporangiophores at either end of the fragment is presumably due to a change in their number as we pass from one whorl to the next. To judge from the rest of the section the sporangiophores were, as in most Calamarian cones known to us, superposed, except where a change in their number led to a disturbance of this arrangement. Secondly, the bracts were more numerous than the sporangiophores : there seem to have been about three bracts to every two sporangiophores. Thirdly, the bracts appear to alternate, though not quite regularly, from one whorl to the next. Fourthly, there is no constant relation, either of superposition or of alternation, between sporangiophores and bracts or between groups of two sporangiophores and three bracts : the two kinds of organ seem to be

inserted on the axis on independent systems. Fifthly, the fact that the numer-
ous sporangia, spread over the considerable portion of the cone traversed by
the section, show only one kind of spore indicates that the cone was homo-
sporous, a conclusion borne out by the presence of but one kind of spore in
the numerous sporangia traversed by the three other longitudinal sections
of the series.

The section shown in Fig. 4 of Pl. IX, which, like that shown in
Fig. 3, does not include any part of the axis, shows considerable por-
tions of two fertile and two sterile whorls, as well as two bracts of a third
whorl and small portions of sporangia at either end of the section.   The
seven bracts in the lowest whorl appear to be coherent, which would seem to
indicate that, at least at this level, this section is more deeply seated than
that represented in Pl. IX, Fig. 3.   The coherence of the bracts is remarkable,
for their freedom in the genus *Calamodendron* is implied by Renault when,
in describing the cone he attributes to his *Arthropitus borgiensis*, he says
that it consists, as do those of *Calamodendron*, of whorls alternately fertile
and sterile, but that the bracts were basally united *instead of being free
throughout their whole length* (Renault, 16, p. 133).   In the other whorl
of this section portions of seven bracts are seen spreading over a wider area
than the coherent bases of the bracts of the whorl below.   Six of these
bracts are grouped in pairs, the bracts composing one such pair being still
connected by a narrow neck of tissue.   It is true that the bracts of this
pair appear to be cut obliquely, and it might be thought that the plane of
the section had met the same bract twice.   On the other hand, the bracts
next to this pair on the reader's left are obviously approximated and their
outline is such that they appear to be cut transversely.   This section does
not show at all clearly the relations of alternation or superposition of the
appendages, for the whorls of bracts are cut at different depths and few of
the stalks of the sporangiophores are preserved *in situ*.   From the distribu-
tion of such as are present and of the sporangia, it is clear that the proportion
of bracts to sporangiophores is somewhat higher than in the sections seen
in Pl. IX, Fig. 3, say about 7 to 4.

The other two sections are more deeply seated, for they include por-
tions of the axis.   They are, unfortunately, short and so thick as to make
it almost impossible to establish the course of the bundles.   At one of the
nodes of one of these sections, where only a few bundles are visible, there
appears to be a certain forking of the strands, but the section breaks off so
abruptly above this node that it is impossible to be sure that there was an
alternation of the bundles at the nodes.   The portion of another node seen
in this section and the two nodes of the other section are too thick to show
the course of the bundles.

Most of the bracts are represented by their fibrous portions.   Only one
bract in the whole series of four slides is sufficiently well preserved to show

the structure of the non-fibrous parts. This is the fourth bract of the fourth whorl, counting from the lower end of Pl. IX, Fig. 3. Even in this case the non-fibrous tissues are imperfectly preserved and do not show up in the photograph, owing to their delicacy and light colour. This bract, of which Text-fig. 2 represents a rough diagrammatic sketch, attained, at the height at which it was cut, a width of approximately 1 mm. and a radial depth of ½ mm. As is indicated in the drawing, the bracts seem to have been imbricated, one
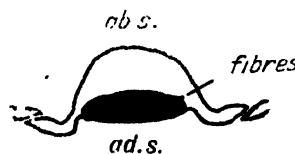
TEXT-FIG. 2. Diagrammatic sketch of a bract of the cone shown in Pl. IX, Fig. 3, representing a transverse section just above the level at which the bract turns upward. × *circa* 30. The vascular bundle, which was probably situated on the abaxial side of the strand of fibres, and the epidermis have perished, as has also, in all probability, some parenchymatous tissue on the adaxial side of the fibres.

wing of each bract lying outside and the other inside the wings of the neighbouring bracts. No trace of the epidermis or of the vascular bundle remains; the latter was, however, presumably situated just below and adjacent to the fibrous strand.

GENERAL DISCUSSION.

The four longitudinal sections which we have been considering clearly belong to a single species. They show all the characters that we should expect in longitudinal sections of *C. magnae-crucis*, both from our knowledge of the transverse sections of the cone of that species and from Renault's description of his unnamed cone of a *Calamodendron* from Grand'-Croix, with which *C. magnae-crucis* is obviously specifically identical. The internodes are of the same length. In Pl. IX, Fig. 3 bracts and sporangiophores are present in the same numerical proportions, though in Pl. IX, Fig. 4 the numerical proportion of the bracts is very slightly higher. In longitudinal and in transverse sections we have the same curious thickenings of the cells of the sporangial walls, the same numerous very small spores, having the same fallacious appearance of being pluricellular and being in both sorts of section arranged in tetrads still surrounded by the spore mother-cell. In both the sporangia are approximated in pairs round the, axis and in the upper series of sporangia of both the approximated sporangia belong to different sporangiophores. Again, we were able to infer that in *C. magnae-crucis* the bundles alternated at the nodes, while the sporangiophores were probably arranged in superposed whorls, except where a change in number led to irregularities. The alternation of the bracts in Pl. IX, Fig. 3, as well as the structure of the axis in one of the deeper-seated sections, suggests that

the bundles alternated at the node, a character which does not seem to have been common in Calamarian cones.   The most extensive of the longitudinal sections also shows that, except for occasional irregularities, probably due to changes in their number, the sporangiophores of successive whorls were superposed to one another.

On the other hand, these four longitudinal sections vividly recall a longitudinal section figured by Renault as belonging to *Calamodendrostachys Zeilleri* (Renault, 16, Pl. LX, Fig. 3).   Our Pl. IX, Fig. 3 in particular is so amazingly like this figure that for a time, having not yet seen the transverse sections of *C. magnae-crucis*, I thought that it might represent a section from the same specimen cut farther out, beyond the axis.   Every one of the characters just enumerated as common to the four longitudinal sections studied and to *C. magnae-crucis* is found also in Renault's Fig. 3, except that the proportion of bracts to sporangiophores is here slightly less high (apparently about five to four, instead of six to four).   Additional resemblances occur between Renault's Fig. 3 and our Pl. IX, Fig. 3.   Thus, there is the same zigzag outline of the upper and lower series of sporangia, the same superposition of the sporangiophores, occasionally disturbed by a change in their number.

In the face of these numerous similarities, amounting almost to identity, it seems impossible to place the longitudinal section represented in Renault's Fig. 3 of Pl. LX of the Atlas of 1893 and the four longitudinal sections in question in different species.   It is, however, almost equally impossible not to regard these last-mentioned sections as belonging to the type of cone represented by the transverse sections shown in Pl. IX, Figs. 1 and 2 of the present paper.   If we accept both these conclusions we can interpret the confusing data in two ways.   We can consider all the sections figured by Renault under the name of *Calamodendrostachys Zeilleri* (Figs. 3, 4, 5, 6, 7, and 8 of Pl. LX of the Atlas of 1893) and the four longitudinal and two transverse sections described in the present paper as belonging to a single species, in the sense in which the word 'species' is generally used in fossil botany.   In this case the species would, of course, be known as *Calamostachys* (*Calamodendrostachys*) *Zeilleri*, Ren., and the name *C. magnae-crucis* would be superfluous.   Or we can regard *C. magnae-crucis* and *C. Zeilleri* as distinct but allied species, including under the former the longitudinal section figured by Renault as belonging to *C. Zeilleri* (Renault, 16, Pl. LX, Figs. 3 and 4) as well as our two transverse sections (Figs. 1 and 2 of Pl. IX) and the series of four longitudinal sections, two of which are represented by Pl. IX, Figs. 3 and 4 of the present paper.   In this case the name *C. Zeilleri* would be retained for the transverse section figured and described by Renault under that name (16, Pl. LX, Figs. 5, 6, and 7 ; 16, pp. 130–32).

The first view is supported by the numerous and striking similarities

already enumerated between the sporangia and spores of *C. Zeilleri* as described by Renault on the one hand and those now described under the name of *C. magnae-crucis* on the other. Moreover, from a study of the bibliography it would even seem possible that Renault himself may have regarded the two types as specifically identical. Before 1896 he twice referred shortly to cones that he attributed somewhat doubtfully to his genus *Calamodendron* and gave particulars of the unnamed cones from Grand'Croix, which show that these cones are specifically identical with our *C. magnae-crucis* (Renault, 14, p. 252 ; 15, pp. 452-4). In 1896 Renault described and figured *Calamostachys* (his *Calamodendrostachys*) *Zeilleri* for the first time (16, pp. 130-2, and Pl. LX), and after this all reference in his works to other cones of *Calamodendron* cease, except for the statement that some of the rare cones doubtfully attributed to this genus are smaller than *C. Zeilleri*, being about comparable in size to *C. Binneyana*. These smaller, specifically unidentified cones have, of course, nothing to do with *C. Zeilleri* or *C. magnae-crucis*. Thus it remains surprising that in so comprehensive a work as the ' Flore fossile d'Autun et d'Épinac' (16) Renault should have omitted all mention of the unnamed but characteristic cone from Grand'Croix in that very district. Portions of the descriptions of the spores and sporangia of *C. Zeilleri* in the account of 1896 are verbally identical with the description of the same structures in the earlier account of the unnamed cone from Grand'Croix. Renault may well have had cognizance of Roche's smaller example of the latter cone with only fourteen sporangiophores, for the two men knew one another. Had Renault come to suspect that the cones possessing sixteen sporangiophores were larger specimens or portions of larger regions of *C. Zeilleri*, and did he, therefore, refrain from giving them a name? The dimensions and proportions of the cones and their constituent parts, given in tabular form below, show that such a view is not untenable, though the number of bracts in a whorl and of bundles in the axis must have presented a difficulty to him. As a matter of fact, although Renault labelled the sections shown in Fig. 1 as having twenty-four bracts, and though this number seems to be the most probable total for the whorl at this level, the slight irregularity in the disposition of the eleven bracts preserved makes it possible to estimate the total as twenty-six or twenty-seven.

| *C. Zeilleri* (*Renault's text and figures*). | *C. magnae-crucis* | | *Characters.* |
	(*Section shown in Pl. IX, Fig.* 1).	(*Section shown in Pl. IX, Fig.* 2).	
9 mm.	10 mm.	8 mm.	Diameter of whole cone.
1·25 mm.	2·25 mm.	1·75 mm.	Diameter of axis.
27 or 28	24 (26–27 ?)	*circa* 21	Number of bracts in whorl.
14	16	14	Number of sporangiophores in whorl.
2 mm.	*circa* 2·5 mm.	2 mm.	Radial depth of sporangia.

If Renault perceived the possibility of the specific identity of his

unnamed cone from Grand'Croix with *C. Zeilleri* he never accepted it, for he did not modify his description of the latter species. Before accepting this specific identity we must, therefore, briefly consider Renault's account and figures of *C. Zeilleri.*

All our first-hand knowledge of this cone is derived from Renault's two closely similar accounts, illustrated by the same plate (Renault, 16 and 17. In the latter paper the plate is reproduced on a smaller scale). These two descriptions may be summarized as follows :

The cone bore, at intervals of about 4 mm., whorls of twenty-eight sterile bracts, and, midway between these, whorls of fourteen sporangiophores. The axis contained fourteen bundles with large carinal canals and secondary xylem. Each bundle gave off traces to two sterile bracts and, just above the level of the bracts, a small strand destined to a sporangiophore. These fourteen small strands ascended the internode to a level slightly above the sporangiophores and were then deflected into these organs.

Renault's description, though short, is extremely definite. Nevertheless, on comparing his figures with his text, we meet at once with a conflict of evidence. The single transverse section of the whole cone figured by him (16, Pl. LX, Fig. 5) gives an almost diagrammatic view of the fourteen sporangiophores and shows outside them a ring of twenty-seven (not twenty-eight) bracts, cut transversely. This discrepancy is not serious, for the bracts are not quite regularly disposed and the lamina of one may have been broken off below the level of the section. Moreover, there is evidence that in Calamarian cones the number of bracts varied somewhat from one whorl to the next (cf. Hickling, 7, p. 11, for *C. Binneyana* ; Renier, 18, p. 11, for *C. Ludwigi,* and p. 325 of present paper). What is more remarkable is that in Fig. 3 of Renault's same plate, which is said to represent a tangential longitudinal section cut from the *same cone* as the transverse section, the stalks of the sporangiophores lie between whorls of bracts only slightly more numerous than themselves. There seem to be about five bracts to every four sporangiophores. As has already been pointed out in connexion with the comparison of this figure with Pl. IX, Fig. 3 of the present paper, the bracts of successive whorls appear to alternate. Renault's Fig. 4 of the same plate represents, on a larger scale, a tangential longitudinal section involving portions of two sterile and one fertile whorl. In it the bracts seem to be equal in number to the sporangiophores and superposed to one another. This figure is, however, probably inaccurately drawn; for, although in the explanations accompanying the plate it is said to represent an enlarged portion of Fig. 3, the latter does not include any fertile whorl with the sporangia disposed as in Fig. 4.

I thought at one time that Renault's figures of longitudinal and transverse sections of *C. Zeilleri* could be harmonized by supposing that a forking of the bracts occurred below the level of the sporangiophores, combined

with the breaking off of one bract tip. Failure to recognize this forking would, I supposed, have led Renault to overestimate in his text the number of bracts. Such a view seemed to receive a certain support from a comparison with the figures illustrated by Pl. IX, Figs. 3 and 4 of the present paper, for were not the bracts in the latter figure almost twice as numerous as the sporangiophores and still approximated in pairs? But this interpretation will not bear closer investigation. In the first place, the bracts in Pl. IX, Fig. 3 of the present paper and in Renault's Fig. 3—which on this view represent the undivided bracts—are rather more numerous than the sporangiophores; while the bract laminae of our Pl. IX, Fig. 4—which on this view are the products of a division—are rather less than twice as numerous as the sporangiophores. Moreover, the basal fusion of the bracts of the lower whorl in Pl. IX, Fig. 4 shows that these supposedly divided structures were cut nearer to their bases than the supposedly undivided ones seen in Pl. IX, Fig. 3. Probably, therefore, the approximation of the bracts in pairs in the second whorl of Pl. IX, Fig. 4 is due either to pressure during fossilization or, possibly, to the fact that the bracts of a pair received their traces from the same bundle, and being, therefore, closely approximated, became free from one another rather late.

It has already been pointed out that a variation in the number of bracts in a whorl is not uncommon in Calamarian cones. The different proportion of bracts to sporangiophores in the sections shown in Pl. IX, Figs. 3 and 4 (approximately three to two in the former and seven to four in the latter) would not result in an exceptionally high variation in number on the basis of fourteen sporangiophores to a whorl. If we include Renault's Fig. 3 with its lower and his Fig. 5 with its higher proportion of bracts to sporangiophores, we get on this basis the following totals of bracts: Renault's Fig. 3, 18; our Pl. IX, Fig. 3, 21; our Pl. IX, Fig. 4, 24-5; Renault's Fig. 5, 27. This shows a considerable, but by no means an impossible, range of variation. As will be shown later, the same variation of 33 per cent. occurs in the number of bracts found in *Calamostachys Grand'Euryi* (cf. pp. 347-8), while in the much smaller *C. Binneyana* the proportional variation so far recorded is but little less.

But even if we were to neglect Renault's categorical statement that there were twenty-eight bracts in *C. Zeilleri*, and were to explain the different proportion of bracts to sporangiophores in his Figs. 3 and 5 as an extreme example of the tendency of the bracts to vary in number, we should, if we are to include all the sections discussed under a single species, still be obliged to harmonize Renault's account of the stelar structure of *C. Zeilleri* with what we know of the anatomy of the sections shown in our Pl. IX, Figs. 1 and 2. Renault's Fig. 6 seems to show that the secondary xylem in the axis was not confined, as it sometimes was in Calamarian cones, to the nodes, but that a certain amount of it was developed as far up in the internode

as the level of insertion of the sporangiophores. Neither is the section shown in our Pl. IX, Fig. 1 nor in Roche's section (Pl. IX, Fig. 2) is there any indication of secondary xylem, and that although the axis in the former section is wider than that in Renault's figure of *C. Zeilleri.* A still more striking difference is that, according to Renault, the axial bundles of *C. Zeilleri* were equal in number to the sporangiophores, while in the sections attributed to *C. magnaecrucis* they were only half as numerous as the latter organs. That Renault should have doubled the number of bundles in the axis is inherently unlikely; and yet he undoubtedly had a tendency to overestimate the number of axial bundles, and it will be shown farther on that in the case of the forms included by him in his *Arthropityostachys Grand'Euryi* and his *A. Decaisnei* he several times gave the number of the bundles as approximately double what it really was.

Again, it seems impossible to reconcile Renault's account of the bracts of *C. Zeilleri* with the structure of the bracts in Pl. IX, Figs. 1 and 3 of the present paper. The later of Renault's two accounts of *C. Zeilleri* (17) gives three text-figures of, and considerably more information about, the bracts than the earlier one. Unfortunately this later description of the bracts is confused by a slip in nomenclature and, perhaps as a result of this slip, by an apparent confusion between the bracts of two distinct species. Renault's Text-fig. 1 of his paper of 1898 (17, p. 400) is said in the text to represent a transverse section of a bract of *C. Zeilleri*, taken a little above the point at which it turns upwards. By an obvious slip the legend below the figure describes it as a bract of *Arthropityostachys* (instead of *Calamodendrostachys*) *Zeilleri.* The figure shows that at this level the edges of the bract were turned upwards towards the adaxial surface and not flattened and imbricated as in our Pl. IX, Fig. 1 and in our Text-fig. 2. Renault's text-figure shows a fibrous strand lying in the middle of the bract, near to the upper surface. This strand is somewhat hollowed out on the lower (abaxial) side and a portion of the vascular bundle lies in this hollow. The vascular bundle is said to be elliptical and its tracheides to be arranged in radial rows, each row being separated from the next by unlignified cells. Renault not only contrasts the structure of the bract and its vascular bundle as seen in his text-figure with the transverse section of a bract reproduced by him as Fig. 5 of Pl. V of the same publication, but also adds that on comparing these two figures it can be seen that the structure of the vascular bundle changes in passing upwards in the bract, the woody elements becoming reduced to a mere transverse band and the hypodermal strand of fibres becoming flattened and dying out—all this without warning the reader that Fig. 5 of Pl. V represents a bract of *Arthropityostachys Grand'Euryi*, Ren. So far does Renault carry the confusion that in his description of *C. Zeilleri* he refers the reader to the bracts figured by him in Figs. 3 and 4 of Pl. V, which represent typical sections of his *A. Grand Euryi*.

Now, the transverse section of a bract shown in Text-fig. 2 (drawn from one of the bracts seen in Pl. IX, Fig. 3) is not identical in form with those seen in Pl. IX, Fig. 1 of the transverse section attributed to *C. magnae-crucis.* It is somewhat smaller than these and the bundle of fibres is somewhat deeper radially, while the lateral wings of the lamina are rather different in shape. In Text-fig. 2 the middle region of the bract appears to be sunken below the wings, but this is clearly due to the destruction of the delicate tissue outside the fibrous strand, which, in the bract in question, appeared to be superficial. On the whole, however, this bract and those seen in Pl. IX, Fig. 1 are very similar in character and they are imbricated in the same way. It must not be forgotten that the bract seen in Text-fig. 2 was cut rather nearer to its base than were the bracts seen in Pl. IX, Fig. 1, and that the differences between them which have been pointed out above are just such as might be accounted for by this fact, and perhaps also partly by the size of the specimen or the region of the cone involved. On the other hand, the bract shown in Renault's Text-fig. 1 (17, p. 400), which is cut just above the level at which the bract bends upwards, shows the edges still adaxially incurved instead of being imbricated. It is only at the level of the sporangiophores that Renault figures the bracts of *C. Zeilleri* as flat. Here, however, they have become very narrow (about ½ mm. in width), and are widely separated instead of being imbricated as in our Pl. IX, Fig. 1 (cf. 16. Pl. LX, Figs. 3, 5, and 6; 17, Pl. V, Figs. 5 and 6).

SUMMARY FOR PART I.

We may summarize the above discussion by saying : (1) that the section figured by Renault as a longitudinal one of *C. Zeilleri* clearly belongs to the same type of cone as the four longitudinal sections studied in Paris, two of which are shown in Pl. IX, Figs. 3 and 4 ; (2) that these five preparations almost certainly represent sections through a cone of the type seen in Pl. IX, Figs. 1 and 2 of the present paper ; and (3) that the balance of evidence is strongly in favour of the view that these seven sections belong to a species of cone which may appropriately be called *Calamostachys magnae-crucis,* and is distinct from the cone described by Renault as *Calamodendrostachys Zeilleri* and represented in his Figs. 5 and 6 of Pl. LX of the Atlas of 1893 (16). It is particularly unfortunate that a careful search through the slides of the Renault and Roche collections in Paris, and through Renault's slides at Autun, not only did not lead to the identification of the original of Renault's Fig. 5 of *C. Zeilleri* (the only one of the published figures which seems really to belong to that species as described by Renault), but even failed to reveal any section that could reasonably be attributed to *C. Zeilleri.* It is to be hoped that such sections may some day be found, for without them the question of the relation of the sections included under *C. magnae-crucis* to Renault's *C. Zeilleri* cannot be regarded as absolutely settled.

Calamostachys magnae-crucis, n. sp., as defined above, may be described in the following words:

The cone was homosporous and about 10 mm. in diameter. The axis varied in width at least from 1·75 mm. to 2·25 mm. and bore at intervals of about 4 mm. whorls of bracts basally coherent for a very short distance. The bracts of successive whorls alternated with one another, and midway between these whorls were whorls of peltate, quadrisporangiate sporangiophores. The sporangia were 2 mm. or a little more in radial depth and contained numerous very small spores. The sporangiophores of successive whorls were superposed, except where a change in their number disturbed this arrangement. There were usually about three bracts to every two sporangiophores, but the proportion of bracts to sporangiophores was sometimes a little higher than this and probably sometimes a little lower (bracts 18-27; sporangiophores 14-16). The axis contained a ring of collateral bundles with endarch primary xylem; in the internodes the protoxylem perished and was replaced by a canal, and the pith was largely fistular. The bundles seem to have been half as numerous as the sporangiophores and to have alternated in successive internodes. At the level of the nodes each bundle probably gave off traces to about three bracts. The traces of the sporangiophores seem also to have arisen from the neighbourhood of the node, two from each bundle; they pursued a steep upward course through half the internode before passing out into the sporangiophores.

THE AFFINITIES OF *CALAMOSTACHYS MAGNAE-CRUCIS*.

The previous pages have made it clear that if *C. magnae-crucis* and *C. Zeilleri* were distinct species they were much alike in many points. It must be borne in mind, however, that the similarities between them may not have been as numerous as a comparison of Renault's description of *C. Zeilleri* with that of *C. magnae-crucis* in the present paper would suggest. For Renault's account was based partly on the section shown in his Fig. 3 of Pl. LX of the Atlas of 1893 (16), and this has been shown to belong to *C. magnae-crucis*. Thus we do not know how many of the characters attributed by Renault to *C. Zeilleri* were observed only in this or similar longitudinal sections. The length of the internodes of *C. Zeilleri* remains doubtful, since this character can only be observed in longitudinal sections. On the other hand, the peculiar thickening of the cells of the sporangial wall and the small size of the spores should be recognizable in such a transverse section as that figured by Renault (16, Pl. LX, Fig. 5). The most important indication of affinity between the two species is probably the origin in both of the traces of the sporangiophores at the node below. In *C. magnae-crucis* these traces do not seem to have ascended above and been deflected into the sporangiophores, as Renault says they

were in *C. Zeilleri.* But, for reasons explained farther on in connexion with *Calamostachys Grand'Euryi* (cf. pp. 344–5), it is quite probable that the deflexion or absence of deflexion of the traces of the sporangiophores was not a specific character, but one varying with the age or region of the cone involved. The chief differences between *C. Zeilleri* and *C. magnae-crucis* seem to have been that in the former species the axial bundles developed secondary xylem in the internode and were as numerous as the sporangiophores, while in the latter they were half as numerous and without secondary xylem in the internodes. In addition, the bracts of *C. Zeilleri* were much narrower and usually more numerous, both actually and relatively to the sporangiophores, than those of *C. magnae-crucis.* Some sections of the latter species, however, such as the section shown in Pl. IX, Fig. 2, approach *C. Zeilleri,* not only in the proportion of bracts to sporangiophores, but probably also in the actual number of the bracts.

PART II. *CALAMOSTACHYS (ARTHROPITYOSTACHYS) GRAND'EURYI*, REN., AND *C. (A.) DECAISNEI*, REN.

I. INTRODUCTION.

The cones originally described by Renault as *Bruckmannia Grand'-Euryi* and *B. Decaisnei* were regarded by him as male fructifications of phanerogamous Calamariae, probably belonging to the genus *Arthropitys* (11). In his later publications he included both in his new cone-genus, *Arthropityostachys* (16 and 17).

Both cones are clearly of the type now generally known as *Calamostachys,* for the axis bore in succession equidistant fertile and sterile whorls. To judge from Renault's descriptions and figures, his two species must have been much alike. In both the sporangiophores in a whorl are said to be half as numerous as the sterile bracts; in both radiating plates of cellular tissue, equal in number to the sporangiophores, extended downwards from the lower surface of certain of the bracts to the level of, or a little below, the sporangiophores. Again, the structure of the bracts in both types is highly characteristic and apparently identical (cf. 12, pp. 42–3, and 16, Pl. LXII, Fig. 5, with Figs. 9, 10, and 11 of Pl. IV of 12).

Renault seems to have distinguished between his two species chiefly by the number of members in the whorls, but also by the number of bundles in the axis and by the dimensions of the specimens. Accounts of the species are given in no less than seven of his publications (11–17). In all these accounts there are said to be twenty-four bracts and twelve sporangiophores respectively in the sterile and fertile whorls of *C. Decaisnei.* In the accounts of *C. Grand'Euryi* published in 1888

and 1890 no mention is made of the number of members of the whorls, but in all the other descriptions the number of bracts is definitely given as thirty-six and that of the sporangiophores equally definitely as eighteen. Turning to the number of bundles in the axis of Renault's two species we find that the accounts vary a good deal. As regards *C. Decaisnei* we find that in 1876 and in 1878 Renault gave the number of the main lacunae (i. e. of the main bundles) as twelve, with twice as many smaller bundles between them. In the small text-book of 1888 and in the account of 1890 the number of the main bundles is given as only six, and it is said that between each of them there are two smaller strands or lacunae; while in the text-book of 1882 and in the later accounts, those of 1896 and 1899, there are said to be twelve main and twelve smaller, more peripheral lacunae. In the case of *C. Grand'Euryi* we find that in the publications of 1876, 1878, and 1882 the number of the main lacunae (i. e. of the main bundles) is given as being equal to the number of the sporangiophores, i. e. as eighteen, while it is added that between them are twice as many smaller bundles. The next two descriptions, those of 1888 and 1890, speak of the axial bundles as varying in number according to the size of the axis, and the earlier account adds that the bundles are of very various relative dimensions. In the latest accounts, those of 1896 and 1898, there are said to be eighteen main axial bundles and eighteen smaller ones lying outside these. Renault gives the dimensions of his two species as follows: Diameter of the whole cone, 8–9 mm. in *C. Decaisnei* and 11–12 mm. in *C. Grand'Euryi*: diameter of the axis, up to 3 mm. in *C. Decaisnei* and 2·5–3 mm. in *C. Grand'Euryi*; length of the internodes, 5 mm. in *C. Decaisnei* and 5·5 mm. in *C. Grand'- Euryi*.

It is obvious that even if these distinctions are accepted as perfectly reliable there will remain some sections showing only one or none of the characters used by Renault to distinguish between his two species. I propose, therefore, to consider under the same heading all the sections in the collections in Paris and in Autun which clearly belong to either of Renault's species. This method is convenient in that it permits us to consider sections in which the characters preserved do not allow of the specimen being referred definitely to one or other of Renault's two species. Moreover, such a proceeding is justified by the fact that many of the specimens appear to be intermediate in character and dimensions between Renault's *C. Grand'- Euryi* and his *C. Decaisnei*. The bearing of this observation will be considered more fully after the sections have been described (see pp. 347–9).

During visits to Paris and Autun I was able to study no less than twenty-one sections apparently belonging to one or other of Renault's closely similar cones, *C. Grand'Euryi* and *C. Decaisnei*. Only one of these sections is at Autun. There were altogether eleven transverse sections. Five of these passed through or very near to the level of the bractigerous

node, three were cut at the level of insertion of the sporangiophores, while two were cut just below and one just above this level. There were ten longitudinal sections, nine of these being tangential and one radial.

II. STRUCTURE OF THE NODE AS SEEN IN TRANSVERSE SECTIONS.

Pl. IX, Fig. 5 of the present paper is a photograph of the original of which Renault's Fig. 6 of Pl. LXII of the Atlas of 1893 (16) represents a small portion. The section includes half the axis cut transversely in the nodal region, where it must have been about 4 mm. in diameter. As can be seen also in Renault's figure, the axis contains two kinds of vascular bundles. Some, and these are the larger, have at their inner edges very definite, relatively large lacunae—of course representing protoxylem that has perished. Between these main bundles are somewhat smaller strands with an internally situated protoxylem group of five to eight tracheides abutting on the well-developed secondary xylem which at the level of the node characterizes main and small bundles alike. The small groups of protoxylem are slightly, but distinctly, more peripheral than the main lacunae. In most cases two smaller strands intervene between the main bundles. In one case, however, two main bundles seem to have been separated by but a single small group of protoxylem, though at first sight a wide sweep of secondary xylem, in reality radiating from a main bundle, suggests the presence of a second small strand. A little more than half the stele is preserved and five main lacunae are visible. The section is slightly oblique and part of it shows the emission of bract traces, while in other places preparations for the departure of these can be made out. The main bundles seem to give off two traces each, much in the manner indicated in Renault's figure. Five large and seven small strands occupy exactly half the axis, and there is an eighth small strand in the fragment preserved beyond the exact half of the axis. This strand lies very close to the main bundle near the left edge of the photograph, and is peculiar in that its protoxylem has perished and is replaced by a small canal. I estimate the number of bracts at this node as probably thirty-six, for, taking the exact half of the stele, the five main bundles probably gave off ten and the seven smaller bundles eight traces, making a total of eighteen for half the cone.

Although this section is unlabelled I do not think that its attribution to *C. Grand'Euryi* is open to serious question. Firstly, Renault says that at the nodes the woody cylinder is twice as thick as in the internodes, and allowing for this increase in width the specimen agrees in size with *C. Grand'-Euryi.* Secondly, the number of bracts suggests a total of thirty-six for the complete whorl, just the number given by Renault for this species. Thirdly, towards the periphery of the preparation may be seen sections of detached bracts of the type characteristic of this species. Finally, Renault

himself, in his latest accounts of *C. Grand'Euryi*, figured a portion of this same slide as a transverse section of the node of this species. The only serious objection that can be urged against this attribution is that the number of bundles in the axis is less than that given by Renault in his latest and fullest accounts of the cone. In these (Renault, 16 and 17), he speaks of there having been eighteen main and eighteen small bundles in the axis. On the other hand, in 1888 and 1890 Renault himself admitted that in *C. Grand'Euryi* the bundles of the axis varied in number. Further, as will appear in the course of the following pages, he seems to have been inclined to overestimate the number of the bundles—especially of the main bundles—in the axis.

There is in Paris a transverse section of a complete axis which is shown in Pl. IX, Fig. 6 of the present paper. Renault's Fig. 2 of Pl. 22 of the text-book (13) of 1882 represents a small portion of this section. The axis here measures about 3 mm. across and contains nine main bundles or lacunae, two of which are closely approximated and somewhat smaller than the others. These two seem to have arisen by the recent division of a single bundle. Between them there is, of course, no small strand ; but in all the other intervals between the main bundles are two smaller and more peripheral strands. Thus there are sixteen smaller strands. This section does not show any indications of the departure of the traces and appears to have been taken slightly above the nodal level, for where the bracts are seen the section passes through their fibrous strands, which are known to have been situated above the vascular bundle and near to the upper surface of the bract. The bracts are coherent and their number and disposition, where preserved, suggest a total of 'about thirty for the complete whorl. As two of the larger lacunae seem to have been produced by the recent forking of a main bundle we shall get a total of thirty-two bracts, if we attempt to estimate their number on the assumption that, as in the nodal section previously described, the main bundles gave rise to two and the smaller bundles to one trace each. The number thirty would seem to be the lowest which could be reasonably adopted as representing the total number. Nevertheless, Renault has labelled this slide ' 8.   , 16 sporangioph. 32

, 24 bractées. *Bruckmannia Grand'Euryi*, à revoir.' I believe that the explanation of this inscription lies in Renault's mental association of this section with his section of *Calamostachys magnae-crucis* already described and figured (cf. pp. 314–15 and Fig. 1). The two sections were found side by side in a box labelled 'Épis d'Arthropitus'. It will be remembered that this section of *C. magnae-crucis* was labelled by Renault ' 8 lacunes, 16 sporangiophores, 32.   , 24 feuilles, pollen pluricellul.' The first four entries clearly correspond to the similar notes on the section at present under consideration. On the slide of *C. magnae-crucis* they are compre-, hensible, for the section does show eight main lacunae, portions of sixteen

sporangiophores and of thirty-two sporangia, as well as the remains of eleven bracts arranged round a little less than half the axis. But for the corresponding entries on the slide bearing the section shown in Fig. 6 there is no sufficient evidence. The number of main lacunae may, indeed, be held to be typically eight, though one has just undergone division. But the section affords no evidence as to the number of the sporangiophores or sporangia, while the number and disposition of such bracts as are preserved is quite incompatible with there having been as few as twenty-four in the complete whorl. At first Renault seems to have admitted the attribution of this section to *C. Grand'Euryi*, since he figured a portion of it under that name in 1882 (13, Pl. 22, Fig. 2). At this time he seems, however, to have believed that there were eighteen main bundles in the axis of *C. Grand'Euryi*, and, influenced no doubt by the fact that the vascular systems of both sections appeared to consist of eight main and sixteen smaller strands, he associated the two sections in his mind. This preconception probably led to his underestimating the number of bracts originally present in the specimen shown in Pl. IX, Fig. 6, and caused him to transfer to the slide bearing this section information derived from the section of *C. magnae-crucis*, while adding the words ' à revoir ' to the specific name of *Grand'Euryi*. The omission of the figure founded on this section from all accounts published after 1882 is significant of these doubts, the more so that Renault was in the habit of using his figures or portions of them again and again. The association of the section shown in Pl. IX, Fig. 6 with the transverse section of the cone of *C. magnae-crucis* (Pl. IX, Fig. 1) is certainly fallacious, since, as has been shown above, the sixteen smaller strands of the latter species are merely traces of sporangiophores, while throughout *C. Grand'Euryi* the smaller strands are true cauline bundles, persisting through the nodes. There is some evidence that Renault at one time attributed the transverse section of *C. magnae-crucis* shown in Pl. IX, Fig. 1 also to *C. Grand'Euryi*, for in his three earliest accounts of this species he writes that besides the main lacunae the axis contains twice as many small lunulate vascular bundles.[1] There do not appear to be any small lunulate strands in any of the sections of *C. Grand'Euryi*. Among Calamarian cones in the Renault collection the only strands that appear to be lunulate in outline (owing to the plane in which they are cut) are the traces of the sporangiophores of *C. magnae-crucis* ; these are, moreover, twice as numerous as the axial bundles. In the four accounts of *C. Grand'Euryi* published after 1812 all mention of lunulate strands disappears, and it is between this date and 1888 that Renault seems to have realized that the section shown in Pl. IX, Fig. 1 belonged to a species distinct from *C. Grand'Euryi*, for it is in 1888 (14) that he first gives a short account of the cone of a *Calamodendron*

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[1] The smaller strands of the axis of *C. Decaisnei* are also said to be lunulate.

from Grand'Croix to which the cone seen in Pl. IX, Fig. 1 certainly belongs (see Part I).

In spite of the apparent fluctuations in Renault's opinion as to the attribution of the section shown in Pl. IX, Fig. 6, the nature of the coherent bracts seen in it, as well as a comparison of the number of bundles with that in the larger axis seen in Pl. IX, Fig. 5, figured by Renault himself as belonging to his *Arthropityostachys Grand'Euryi*, makes it clear that in the section shown in Pl. IX, Fig. 6 we are dealing with a smaller specimen of the same species of cone.

There are in Paris three transverse sections cut in the neighbourhood of the node, which, though somewhat smaller than the two just described, are similar to them in all essentials. The structure of the stelar tissues suggests that these three sections represent a series of which the members are cut relatively close to one another from the neighbourhood of the same node. This is borne out by the presence on the slides of labels bearing the numbers 29, 32, and 33. The section labelled 29 is wider and possesses considerably more secondary xylem than the other two, and presumably passes nearest to the actual level of the departure of the traces. As the section labelled 32 is endorsed in Renault's writing ' au-dessus du verticille sterile, F. 21 ', that numbered 33 presumably represents the uppermost of the three sections.[1] The axis of the slightly oblique section labelled 29 (Pl. IX, Fig. 7) must have been about 4·25 mm. across. It contains seven main bundles or lacunae, somewhat irregularly disposed. Two of these are markedly smaller and relatively close together, their size and position suggesting that they have arisen by a recent division or are about to fuse. Between these two bundles there are no smaller strands, but in the other cases the main bundles are separated by smaller more peripheral strands with persistent protoxylem. There seem to be twelve smaller strands, but owing to the thickness of the section it is not always easy to distinguish the small groups of protoxylem. The number of small strands intervening between two main bundles varies according to the distance between the latter ; in two cases main lacunae are separated by but a single small strand ; in two cases by two, and in two cases apparently by three such strands. In this preparation the bracts are preserved *in situ* round but a small part of the axis ; their disposition suggests a total of about twenty-six or twenty-eight. The section does not show the actual departure of the

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[1] The slides labelled 32 and 33 are endorsed in Renault's writing ' épi de *Calamodendron* ', while the slide labelled 29 is similarly endorsed ' *Arthropitus*. à photographier '. It may seem strange that, while the structure and numbering of these sections suggests that they represent a series, Renault should have labelled two *Calamodendron* and one *Arthropitus* ; for, whether or no the cones of these two types can be distinguished, he regarded them as generically distinct. The explanation seems to be that though in his published work Renault accepted Goeppert's subdivision (5, p. 183) of Brongniart's genus *Calamodendron* (1, p. 49), he may well in his manuscript notes sometimes have used the name *Calamodendron* in the wider sense in which it was used by his master Brongniart.

traces, but if the proportion of bract traces to bundles was as in the section shown in Pl. IX, Fig. 5, the total number of bracts must have been rather over twenty-six.

The next section of the series, that labelled 32, also contains seven main lacunae and apparently twelve smaller bundles, but it is unfortunately so thick in parts that it is not easy to be sure even of the number of the small bundles. If, as seems likely, Renault's inscription ' F. 21 ' stands for ' Feuilles 21 ', this is an obvious under-estimate of the total number of bracts in the whorl immediately below. The disposition of the closely crowded bracts round the small part of the axis over which they are preserved suggests a total of at least twenty-six, possibly twenty-eight, which is about the number we should have expected from a comparison of the number of bundles in this section and with that shown in Pl. IX, Fig. 5.

The third and uppermost section of the series, that labelled 33, includes a relatively small part of the axis, is markedly oblique, and too thick to show details ; but so far as could be made out it agreed with the last-described section.

III.   THE STRUCTURE OF THE INTERNODAL AXIS OF THE CONE.

Three of the transverse sections of the internodal axis belong to the larger type of cone described by Renault as *Bruckmannia* (later *Arthropityostachys*) *Grand'Euryi*.

One of these sections (Pl. X. Fig. 8) passes radially through the sporangiophores and is the original of which Renault's Fig. 1 of Pl. LXII of the Atlas of 1893 (16) represents half.   The second (Pl. X, Fig. 9) is a section passing through half the cone a little above the level of the sporangiophores and is the original of Fig. 2 of Renault's same plate.   The third of these sections is now preserved in the Musée d'Histoire Naturelle at Autun.   Though it has never been figured and the slide bearing it is merely labelled ' Débris divers ', it may safely be attributed to *C. Grand'Euryi*.   It shows in places fragments of the peculiar radiating plates of cellular tissue characteristic of this species and of *C. Decaisnei*, Ren., while the disposition of the sporangiophores, suggesting a total of eighteen for the complete whorl (though sixteen or seventeen would be possible numbers), and the diameter of the axis (3·25 mm.) indicate that the section belongs to the larger of the two forms, although the whole cone appears to have been only about 8 mm. in width at this level.   As this section, now at Autun, is from the same region of the internode as, and is much less well preserved than, the section of the complete internodal axis in Paris, the following description is, except where otherwise stated, based on the sections in Paris.

Although the five publications in which Renault mentions the number of sporangiophores in the whorls of *C. Grand'Euryi* all give this number as

18, yet his Fig. 1 of Pl. LXII of the Atlas of 1893 (16), which represents half a cone in transverse section, shows six sporangiophores with portions of two others. This suggests a total of but fourteen for the complete whorl, and in the complete section, now figured (Pl. X, Fig. 8), it can be clearly seen that there were fourteen sporangiophores arranged very regularly round the axis. The next section (Pl. X, Fig. 9) passes somewhat above the sporangiophores through the radiating cellular plates that are known from longitudinal sections of the cone to have extended downwards from the bracts to the sporangiophores. It is remarkable that, whereas Renault's figure of this section (16, Pl. LXII, Fig. 2) shows only seven of these plates, the original passes through nine, two of which are not shown quite in their entirety. There were thus probably seventeen or eighteen cellular plates in the section of the complete cone, so that the original section is in fair agreement with Renault's statements that there were eighteen sporangiophores and that the cellular plates were equal in number to these; whereas his figure of it leads to the deduction that there were fourteen cellular plates and therefore fourteen sporangiophores in the whorl just below the section. Turning from Renault's figures to the original sections, we see at once (Pl. X, Fig. 8) that at the level of insertion of the sporangiophores the bundles of the axis are not sharply distinguished, as they are at the level of the bractigerous node, into main bundles with relatively large canals and much smaller, more peripheral strands with persistent protoxylem. In the section represented in Pl. X, Fig. 8 the axis contains eighteen canals, no doubt corresponding to protoxylem that has perished. Some of these canals are relatively large and deep-seated, others very small and more peripheral; but there are yet other canals intermediate in size and position between these extreme forms. It is possible, however, to regard six canals as corresponding to the main bundles of the node, for all of these are of considerable size and none of them occupies a relatively peripheral position. If we imagine a vertical line passing down the middle of the stele, as seen in the photograph, and number the sporangiophores one to fourteen, beginning with that on the right of this line, we find that of the six main lacunae one lies opposite the ninth sporangiophore, while the others are situated respectively between the second and third, between the fifth and sixth, between the seventh and eighth, between the eleventh and twelfth, and between the fourteenth and first sporangiophores. Of the twelve canals regarded as belonging to the smaller bundles, some, such as those opposite to the first and second sporangiophores, are very small and lie so far out as to suggest, at first sight, departing traces. Others, such as those opposite to the fourth and thirteenth sporangiophores, are much larger and rather more deeply seated, though still somewhat more peripheral than the main lacunae. In fact there are, as may be seen from an examination of Pl. X, Fig. 8, a number of intermediate sizes of strands between the extreme types

of small and large strands. The figure also shows that the vascular strands, large and small alike, are not quite regularly arranged. A comparison of this photograph with Renault's drawing of the section from which it was taken shows that his whole figure is too diagrammatic, and that, in particular, he gives too regular and quite inaccurate a representation of the distribution of the lacunae in the axis.

The section (Pl. X, Fig. 9) representing half a cone cut just above a fertile whorl includes the whole of two main lacunae and rather more than half of two others, so that there are probably six, possibly seven, main bundles in the complete axis. This section also shows in a more peripheral position six smaller strands, one of which is markedly larger than the others. Owing to the thickness of the section, two of the smallest canals are indistinct in the photograph. The complete axis presumably possessed twelve of these smaller strands. Renault's figure of this section is again inaccurate (16, Pl. LXII, Fig. 2). He has introduced more canals than the section warrants, and in showing the bracts as though they were arranged regularly round the axis he has drawn largely on his imagination.

In both sections it appears to be the rule—though not the invariable rule—that the main lacunae lie not opposite to, but between, certain of the sporangiophores, usually between the bases of every two of them. This is true of all the main lacunae seen in Pl. X. Fig. 9 and in five out of six of those seen in Pl. X, Fig. 8. Though the section of *C. Grand'Euryi* at Autun is rather fragmentary, the distinction between the two kinds of canals—the smaller, more peripheral ones situated opposite to the sporangiophores and the larger, deeper-seated ones apparently lying between the bases of certain sporangiophores—seems to be more sharply marked in it than in the section represented in Pl. X, Fig. 8.

The three remaining internodal transverse sections clearly belong to the smaller form of cone described by Renault as *C. Decaisnei*. One of these smaller axes (Pl. X, Fig. 10) is the original of Fig. 12 of Pl. IV of Renault's paper of 1878 (12)—one of the only two published figures of *C. Decaisnei*.[1] It has been labelled by Renault '*B.* (i. e. *Bruckmannia*) *Decaisnei*', and passes through the cone at the level of the sporangiophores, some of which are cut radially. Allowing for compression the diameter of the cone must, at this level, have been about 8 mm., and that of the axis about 2·25 mm. On comparing Pl. X, Fig. 10 with Renault's figure, it is easy to recognize the main features of the section. Renault's drawing is, however, idealized, for it shows a network of cells, whereas the section is far too thick to show the outline of the individual cells. At the edge of the section are the remains of many of the bracts, not all of which are included in the photograph. Though they are not arranged quite as regularly as Renault's figure

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[1] The other figure, Fig. 13 of the same plate, represents a small fragment of a longitudinal section, the original of which has not been identified.

suggests, they do seem to have been twice as numerous as the sporangiophores, and, if so, there would have been, as he says, twenty-four in the whorl. As to one point Renault's figure is less clear than the original section. In it neither the number nor the position of the canals is well seen, and they are not sharply distinguished into large and small. In the original section (Pl. X, Fig. 10) there are six main lacunae lying between the bases of every two sporangiophores. On one side of the photograph two smaller canals may be seen, lying outside and between the main lacunae. As big and small canals are equidistant, the smaller ones are opposite to sporangiophores. On the other side the section is slightly oblique and does not show definite small lacunae, though in one place there are indications of a small canal. To judge from the other two small axes, there were usually two small strands between each of the main lacunae. Renault expressly states that the canals of the smaller bundles are often indistinct (14, pp. 239–40; 15, p. 451; 16, p. 135). It is, therefore, probable that the absence of the small canals on one side of the photograph is due to their being obscured by the obliquity and thickness of the section in this region, or possibly to the persistence of the protoxylem of some of the small strands.

Both the other sections of small axes were unlabelled and were found in different boxes, but their stelar structure is so similar to that shown in Pl. X. Fig. 10 that they clearly belong to the same type of cone. The absence of any remains of the cellular plates found in the upper half of the internode indicates that they were taken from below the level of the sporangiophores. One of them (Pl. X. Fig. 11) had a diameter of a little over 2 mm. and contained eighteen equidistant bundles, six main and twelve smaller, all provided with an internal lacuna. The pairs of smaller strands intervening between the main bundles are more peripheral in position, and lie opposite to slight prominences of the axis, almost imperceptible on one side of the section, while the main bundles lie between every two of these. The slight prominences obviously represent the bases of or lines of attachment of the sporangiophores. The arrangement of the fragments of the peltate heads of three sporangiophores visible near the edge of the photograph is consistent with there having been twelve sporangiophores in the whorl immediately above.

The third of these transverse sections of smaller internodal axes is less well preserved, but agrees in all essentials with that just described and shown in Pl. X, Fig. 11. In particular, pairs of small strands, provided with small canals, occur between and somewhat externally to such of the main lacunae as are preserved.

IV. Superposition or Alternation of the Whorls of Appendages.

Apart from disturbances due to changes in number, the bracts and sporangiophores seem to have been superposed respectively to the similar organs of the whorls above and below. This is usually well seen in tangential longitudinal sections through the peripheral regions of the cone. Where the section involved more than one whorl of the same sort, the superposition of bract to bract or of sporangiophore to sporangiophore was usually very clear. Indeed, considering the variation in the number of members in the whorls' observed in transverse sections of these cones, the superposition is surprisingly constant, and rather suggests that the transverse sections studied belonged either to different cones or to widely separated regions of the cone.

If we want to study the relations of the sporangiophores to the bracts, tangential longitudinal sections again supply the clearest evidence. Though Renault's figures of tangential longitudinal sections of the cone of *C. Grand'-Euryi* (16, Pl. LXII, Figs. 3 and 4) are to a certain extent restorations, yet they reproduce accurately the main features of the portions of the sections which they represent. Though the bracts appear to be about twice as numerous as the sporangiophores, there is no accurate superposition or alternation between the latter organs and the bracts or pairs of bracts. This is true of all the tangential longitudinal sections which are sufficiently extensive to allow of a study of this character. It is, of course, obvious that where a large number of bracts are regularly disposed on a narrow axis that also bears, higher up, about half as many sporangiophores, the radial lines of insertion of the latter organs will be but little removed from those of the neighbouring bracts. But, even apart from disturbances due to changes in number, there is, apparently, no regular alternation or superposition between the two categories of organs. An examination of the transverse sections confirms this view. Even in the section shown in Pl. X, Fig. 10 of the present paper, where the vascular cylinder is of a more regular type and where the bracts were probably exactly twice as numerous as the sporangiophores, the bracts are not disposed regularly on either side of the median line of the sporangiophores, as they are in Renault's figure of this section (12, Pl. IV, Fig. 13), while in his drawings of the sections shown in our Pl. X, Figs. 8 and 9 the regular disposition of the bracts (16, Pl. LXII, Figs. 3 and 4) is entirely imaginary. In fact, all the evidence available tends to show that bracts and sporangiophores are inserted on the axis on systems independent of one another.

V. The Course of the Axial Vascular Bundles.

Renault makes a very surprising statement about the course of the vascular bundles in the axis of *C. Grand'Euryi.* He says (16, p. 136) that each of the woody wedges (our vascular bundles) gives off traces to two bracts 'after having divided into two branches, as occurs at each articulation of the stem (Fig. 6)'. This statement is in absolute contradiction to his Fig. 6 of Pl. LXII of the Atlas of 1893 (16), with a reference to which the sentence concludes. That figure, which represents part of the section shown in our Pl. IX, Fig. 5. shows, as does the original, an undivided bundle giving off two traces. Pl. IX, Figs. 6 and 7 of the present paper show that branching of main bundles did occur at or near the node, but only occasionally. Most of the bundles, however, were continuous through the nodes. It would be surprising if there were a regular alternation of the bundles at the node, since the bracts of successive whorls are superposed to one another. At the same time the occasional variation in the number of bracts in the whorls might well be correlated to a limited anastomosis of the bundles, while it is also possible that the absence of a definite relation, either numerical or of position, between bracts and sporangiophores led to an occasional anastomosis. Owing to the crowding of traces and bundles and the increased width of the axis at the nodes, the course of the vascular bundles cannot be well followed in longitudinal sections of the axis of *C. Grand'Euryi* (cf. Pl. X, Figs. 12 and 13, and Pl. XI, Fig. 14).[1] The disappearance of the canals at the level of the uppermost node seen in Pl. X, Figs. 12 and 13 seems to be due, not to the alternation of the bundles, but to a change in the relative depth of the section, due partly to its obliquity and partly to the increased width of the axis at the node. That this is so is shown by the change in outline of the central cavity, and by the fact that the canals left by the protoxylem are only visible throughout part of the lower internode.

Two longitudinal sections of the smaller or *C. Decaisnei* type of cone are sufficiently deep seated to show something of the course of the axial bundles. The first of these (Pl. XI, Figs. 15 and 16) is a very fine specimen. Owing to the kindness of the authorities of the Paris Museum it was ground thinner for me, though even now it remains rather opaque. The portion preserved includes four nodes with part of a fifth, three complete internodes with considerable portions of two more. The section is slightly oblique, and becomes nearly radial in its upper part. To judge from this part of the section the axis was probably a little less than 2·5 mm. in width, while

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[1] The attribution of the section shown in these figures to *C. Grand'Euryi* is based on the fact that the number of bract traces and of bundles is greater than would be visible in a tangential longitudinal section of *C. Decaisnei.* As to the impossibility of distinguishing with any certainty between these two species, see pp. 347-9.

the diameter of the whole cone was about 8 mm. The internodes are exactly 5 mm. long. These dimensions, combined with the remains of cellular plates attached to the sporangiophores, suffice to identify the specimen with Renault's *C. Decaisnei.* In the lower, more tangential part of the section, two main bundles, represented by their canals, can be seen. In this section the smaller canals which presumably intervened between these two main canals are not seen, doubtless because the smaller strands were more peripheral in position. Towards either edge of the portion preserved, where the section naturally passes nearer to the periphery of the axis, may be seen indications of smaller lacunae (cf. Pl. XI, Figs. 15 and 16). The short streak lying between the two main bundles above the second node of Pl. XI, Fig. 15, which at first sight suggests a lacuna, presumably represents the outer edge of the central cavity, since it lies where the plane of the section is most deeply seated. In this section neither main nor small bundles alternate at the nodes. It is true that above the third node the canals seem to disappear, but the appearance and outline of the central cavity indicate that the section here passes more internally, so that its plane no longer coincides with the canals of the vascular bundles. Through the second node the continuity of the bundles is very obvious, doubtless because the section is here more truly longitudinal. This continuity is well seen in the radial longitudinal section shown in Pl. XI, Fig. 17. The slide bearing this section has been endorsed by Renault 'contracté aux nœuds'. The axis is not contracted at the nodes, though the central cavity is, very slightly. Only one side of the axis and two nodes are seen in the photograph. The internodes are about 5 mm. long, and the woody zone is less thick than in most of the cones described or figured by Renault. With the exception of the base of a sporangiophore the section does not show the attachment of any appendages, though detached fragments of the cellular plates and of the bracts characteristic of *C. Grand'Euryi* and *C. Decaisnei* occur. Near the upper node is a portion of a bract which, though not actually in connexion with the axis, is only slightly separated from it. This bract seems to have been pushed downwards by the large black mass which overlays it, but has not been much displaced. At a distance of about 2 mm. from the axis it turns upwards, thus giving a clue to the orientation of the section.[1]

It is not surprising that neither of the longitudinal sections showing the course of the bundles in the smaller or *C. Decaisnei* type of cone should afford any evidence of occasional anastomosis of the bundles at the nodes. For we saw that in the larger or *C. Grand'Euryi* type such occasional anastomosis was probably correlated to a change in the number of the appendages, and the sections through the smaller cones just described seem to show that in the *C. Decaisnei* type of cone the structure of the cone was

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[1] This orientation is confirmed by the course of the trace in the sporangiophore (cf. p. 343).

more regular and the number of appendages in the whorls more constant than in the larger type. At the same time the number of preparations showing the course of the bundles in the smaller type of cone is quite insufficient to justify the generalization that in such cones the bundles were always continuous. In this connexion we may note that though Renault stated, apparently erroneously, that in his *C. Grand'Euryi* the bundles alternated regularly at the nodes, and though he made no statement as to their course in *C. Decaisnei*, he never suggested, when summarizing the differences between the two types, that there was any difference in the course of the axial bundles.

VI. The Course of the Traces of the Sporangiophores.

There appears to be some confusion as to the meaning of Renault's account of the course of the traces of the sporangiophores and their relation to the axial bundles in *C. Grand'Euryi*. Renault's statement is to the effect that in this species the eighteen smaller and more peripheral bundles of the central cylinder enter into connexion with ('se mettent en rapport avec') the strands which penetrate the sporangiophores in the manner already described ('à la manière que nous avons déjà exposée') (16, p. 136). Some botanists appear to have thought that these last words refer the reader to the description of the course of the traces of the sporangiophores in *Calamostachys* (*Calamodendrostachys*) *Zeilleri* (7, p. 12; 19, p. 53), in which species they are given off at or near the node below and pass upwards through more than half the internode, then descending somewhat and entering the sporangiophores.[1]

It does not seem to me that Renault's words should be construed in this sense, for between the accounts of his *Calamodendrostachys Zeilleri* and his *Arthropityostachys Grand'Euryi* are intercalated accounts of two other of his types of cone (*A. borgiensis* and *A. Decaisnei*). Renault's words might perhaps be held to mean that the traces of the sporangiophores departed in the manner already described for *C. Grand'Euryi* on an earlier occasion. If so, the reference would be to his paper of 1878, since that is the only publication of his mentioned in the account of *C. Grand'Euryi* in which the words in question occur. But in the paper of 1878 (12) the course of the sporangiophore traces of *C. Grand'Euryi* is only described in a footnote, in which it is said that each sporangiophore receives traces from two neighbouring bundles. This clearly erroneous statement is corrected in all subsequent accounts, except that of 1882 (13), and is, moreover, in flagrant contradiction with Renault's statements in the publication from which the words quoted are taken. Presumably, therefore, Renault meant to imply

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[1] Though the fact was not mentioned by Renault, the traces of the sporangiophores of *C. magnae-crucis* seem also to have originated at or near the node below.

that in *C. Grand'Euryi* the traces penetrate the sporangiophores in the manner already described in the immediately preceding account in the same paper, of the very similar cone, *C. Decaisnei.* In this account the trace of the sporangiophore is said to have its departure in the portion of the internode situated immediately above these organs (16, p. 135).

Two of the longitudinal sections that have already been discussed in treating of the course of the vascular bundles throw some light on the course of the traces of the sporangiophores in the smaller or *C. Decaisnei* type of cone. The section of which Pl. XI, Fig. 15 is a photograph is rather thick, but it nevertheless shows indications of tracheides sweeping outwards and downwards into the bases of certain of the sporangiophoies. This downward sweep of the trace of the sporangiophore is best seen in Pl. XI. Fig. 16, which represents a portion of the same section on a larger scale. The tracheides passing downwards on their way to the sporangiophores can be made out on the reader's right near the upper end of the figure. Although the scale of Pl. XI, Fig. 15 is too small to show the details at all clearly, a similar downward deflexion of the trace can just be made out in connexion with the base of the uppermost sporangiophore in the left-hand corner of the photograph. The downward deflexion of the trace is, however, much better seen in the next figure, Fig. 17. This represents the radial longitudinal section already mentioned and shows a trace of a sporangiophore which is deflected downwards for a distance of about 400 μ. The justification for the orientation of this section has already been discussed, but further support for it is found in Renault's statement that in *C. Grand'Euryi* and *C. Decaisnei* the vascular strands traverse the upper parts of the sporangiophores (12, p. 44, repeated for *C. Decaisnei*, 15, p. 451). By this statement Renault presumably meant that the vascular strand ran along the upper side of the stalk of the sporangiophore with reference to the whole cone. Pl. XI, Fig. 17 shows that this was so, at least at the proximal end of the stalk, if the section is oriented as in our figure. The fact that the only transverse section of Renault's *C. Decaisnei* actually passing through the sporangiophores which has been identified (our Pl. X, Fig. 10) does not show the departure of any traces might also be quoted to support the view that these were given off above and deflected downwards into the sporangiophores. This section is, however, so thick that I do not think much stress should be laid on the absence of any indication of the departure of traces.

While it has been shown above that Renault's statement that in *C. Grand'Euryi* the traces penetrate the sporangiophores in the manner already described probably means in the manner already described for *C. Decaisnei*, there is less direct evidence as to the course of the traces in the larger or *C. Grand'Euryi* type of cone. There is no longitudinal section showing the origin of the traces of the sporangiophores in this type. In the single transverse section of the axis passing through some of the sporangio-

phores, now in the museum at Autun, no traces are visible and the circular outline of the main and smaller canals suggests that their emission occurred at a higher level. Though the section represented in Pl. X, Fig. 8, one of Renault's type specimens, does not show the departure of any traces, some of the canals lying opposite to the sporangiophores project outwards as though about to give off a branch of protoxylem, while others, also opposite to sporangiophores, remain circular in outline. The outline of these canals, combined with the absence of departing traces and with the presence of a small canal within the base of one of the sporangiophores, certainly suggests that the traces of the sporangiophores did not pass out horizontally. On the other hand, the canals which appear to be about to give off branches to the sporangiophores suggest that, if there was a deflexion of the traces, it was probably slight. This is confirmed by the section shown in Pl. X, Fig. 9, the plane of which is only slightly above the level of the stalks of the sporangiophores, since it shows towards the exterior the radiating cellular plates above these and, abutting on the axis, the bases of the stalks. As no traces are to be seen and as the canals are circular in outline the emission of the traces appears in this case to have been already completed.

It is quite probable that there was a good deal of variation in the course of the traces at different levels of the cone and in cones of different ages. Although the cone of *Equisetum maximum*, Lam., is very different in structure from *Calamostachys Grand'Euryi*, it is quite comparable in size with the latter, and in large specimens the lower whorls, at any rate, consist of as many or of more sporangiophores. The distance between the whorls is, however, less, a difference probably correlated to the absence of sterile bracts in the recent plant. The distance between successive whorls of sporangiophores seldom, if ever, attains 4 mm., and even in fully mature basal regions of the cone is often only 2 mm. Nevertheless, the traces of the sporangiophores of the lowest whorls of mature cones are usually much more markedly deflected in passing through the cortex than any of the traces of the fossils under consideration. In some cases the downward deflexion was as much as 1·5 mm., while in the case of a large mature cone the *average* downward deflexion of the traces of the lowest whorl was 0·997 mm. In other parts of this same cone the traces pass out horizontally, or even, in the upper regions, pursue an obliquely ascending course. In *E. maximum* the degree of deflexion of the sporangiophores appears to be largely dependent on their level in the cone and on the degree of maturity of the latter. The traces of the lower and more mature regions show, as a rule, progressively greater deflexion (Browne, 3, pp. 243-7). Consequently, while it seems probable that some deflexion of the traces of the sporangiophores occurred in *C. Grand'Euryi*, as it certainly did in *C. Decaisnei*, yet I do not think that it follows that a deflexion of the traces

was general throughout the cone or should be regarded as a specific character of either of Renault's types.

We may now pass to the question : Which were the bundles that gave off traces to the sporangiophores ? In the more regular, smaller types corresponding to Renault's *C. Decaisnei* it would seem natural to suppose that the twelve smaller and more peripheral strands that lie opposite to the twelve sporangiophores gave off traces to them. All Renault's publications from 1888 onwards state that this is so, and the hypothesis is confirmed by the radial longitudinal section now figured (Pl. XI, Fig. 17), in which a trace is passing out to a sporangiophore from a bundle of slight radial depth, even for one of the smaller strands. For the larger types, corresponding to his *C. Grand'Euryi*, Renault's statement that the traces of the sporangiophores were inserted on the smaller, more peripheral bundles cannot be unreservedly accepted. ' These bundles are not as numerous as Renault supposed. Certainly in some, and probably in most, cases they did not supply all the sporangiophores with traces. In the section shown in Pl. X, Fig. 8, one of Renault's type sections, it can be seen that the twelve smaller strands [1] lie more or less opposite to twelve sporangiophores and no doubt supplied them with traces, though not all of these small strands show preparations for the emission of traces. Probably, therefore, the traces of the sporangiophores of the same whorl sometimes departed from slightly different levels, as is often the case in *Equisetum maximum* (3, Pls. XII and XIII). This seems to be the principal factor in the variation in size of the canals of the smaller strands at this level. On the other hand, the canal opposite the ninth sporangiophore seems from its size and position clearly to belong to a main bundle, and it is certainly preparing to give off a trace. Opposite to the fourteenth sporangiophore there seems to be no small strand, so that the trace of this organ was probably given off from the main bundle lying between the fourteenth and first sporangiophores. Owing to the thickness and slight obliquity of the section in this region it is difficult to make out the stelar structure, and it is just possible that there was at this point an additional small strand, or that such a strand may have passed out in its entirety. The latter possibility is suggested by the unusually peripheral position of the small strand opposite to the seventh sporangiophore, and it may be well to recall in this connexion that the passing out of a vascular strand in its entirety into a sporangiophore is frequently the means by which the number of axial bundles is reduced in the cone of the recent genus *Equisetum* (2, p. 677 ; 4, pp. 435–7). On the other hand, Pl. X, Fig. 8 suggests and the transverse sections of the node (Pl. IX, Figs. 5, 6, and 7) show that certainly most and probably all of the smaller bundles usually persisted through the nodes from one internode to the next.

[1] For the not very obvious distinction of the bundles of this section into large and small, see p. 336.

The conclusion seems to be that in the larger type, that of *C. Grand'-Euryi*, each smaller bundle was situated opposite to a sporangiophore, to which it gave off a trace, and that most of the main bundles lay between certain of the sporangiophores and took no part in the emission of traces to these; but that a few (one or two) of the main bundles occasionally lay relatively near to the points of insertion of sporangiophores to which they seem to have given off traces. The variation in the number of sporangiophores in the whorls doubtless led to an opportunist method of vascular supply. Usually the smaller and more peripheral bundles were opposite to the sporangiophores and supplied them with traces, but where the smaller bundles were not as numerous as the sporangiophores the vascular supply of the latter was taken over by the main bundles nearest to them.

VII. The Structure of the Bracts.

The bracts were basally fused into a coherent disc which extended horizontally for a distance of from $2\frac{1}{2}$ to over 3 mm. (cf. Pl. IX, Figs. 6 and 7). In this horizontally extended portion the hypodermal fibrous strand lying near to the upper surface occupies almost the whole width of the bract. From the lower surface of this disc a series of radiating, parenchymatous plates, equal in number to the sporangiophores, extended downwards to these organs, passing between their two upper sporangia (cf. Pl. X, Fig. 9. and Pl. XI, Fig. 15). According to Renault the bracts sometimes extended upwards through two complete internodes (16, p. 136; Pl. LXII, Fig. 2). If so, their length, including their fused bases, must have been over 13 mm. When the bracts became free their edges were involuted against the adaxial (morphologically upper) surface of the lamina (cf. 16, Pl. LXII, Fig. 5 of the Atlas of 1893). At this level the bract, as seen in transverse section, is markedly convex adaxially in its median region. The hypodermal fibrous strand, now occupying a smaller proportion of the width of the bract, lies in this convexity, near to the upper surface. The vascular bundle was situated below and in contact with this strand.

VIII. The Distinctions between *C. Grand'Euryi* and *C. Decaisnei*.

The criteria upon which Renault relied to distinguish between *C. Grand'Euryi* and *C. Decaisnei* were of two kinds: those founded on the dimensions of the specimens and those based on the number of bundles in the axis or of members in the whorls. The distinctions founded on the dimensions depend (1) on the diameter of the cone (10–12 mm. in *C. Grand'-Euryi*, 8–9 mm. in *C. Decaisnei*); (2) on the diameter of the axis (2·5 mm.– 3 mm. in *C. Grand'Euryi* and up to 3 mm. in *C. Decaisnei*); (3) on the

length of the internodes (5·5 mm. in *C. Grand'Euryi* and 5 mm. in *C. Decaisnei*). The first distinction can be observed in transverse sections if they are sufficiently extensive and include remains of bracts *in situ*; but is often not to be seen in longitudinal sections, as the plane of these may pass between the bracts. The diameter of the axis is only decisive when it is under 2·5 mm. and cannot be observed in the more peripheral longitudinal sections. The third character, the length of the internodes, cannot be observed in transverse sections. Again, the cones of *C. Grand'Euryi* were, according to Renault, 7–8 cm. long, so that, apart from a certain variation in the size of individuals, the younger cones and the apical portions of cones were, presumably. somewhat smaller than typical sections. A difference of 0·5 mm. in the length of the internodes and of 1 mm. in the width of the whole cone seem, therefore, to be insufficient and arbitrary reasons for separating otherwise closely similar types.

The characters founded on the number of bracts and sporangiophores in a whorl and of bundles of the axis seem at first to be of greater importance. But in longitudinal sections they can only be observed in those tangential ones that show too many bundles, traces. and appendages for the specimen to belong to *C. Decaisnei.*

The principal difficulty of the problem, however. is that Renault's criteria, when applied, often gave contradictory results. Thus, from the number (7) of bract traces seen in Pl. XI, Fig. 14, the section it portrays would seem to have belonged to *C. Grand'Euryi*, in which, according to Renault, the length of the internodes is 5·5 mm. Yet in it the internodes do not attain quite the 5 mm. which are said to be characteristic of *C. Decaisnei.* If we turn to the characters based on the number of bundles or of members in the whorls, Renault's distinctions break down even more completely. Table I contains particulars of these characters extracted from Renault's seven papers dealing with *C. Grand'Euryi* and *Decaisnei.* Table II supplies similar particulars, so far as they could be ascertained. from a study of the transverse sections in Paris, that at Autun being left out of account because of its fragmentary condition. Where the section does not actually show the full number of members in a whorl, or of bundles in the axis, but allows this number to be deduced with fair accuracy, the figure given is followed by the letter (e) to show that it is an estimate. The facts on which these estimates are based will be found in the description of the sections in the earlier part of the paper.

It will be seen that the only sections in Table II in which all the characters observed can be harmonized with either of Renault's types are the last three. These seem definitely to belong to Renault's *C. Decaisnei.* It is true that they differ from this species, as described in most of his accounts, including the two latest, in that the axis contains six and not twelve main bundles. In this, however, they agree with the accounts given

in 1888 and 1890. Moreover, these three sections agree so closely with Renault's *C. Decaisnei* that even had he not labelled one of them, that shown in Pl. X, Fig. 10, their attribution to this type would be obvious. The sections shown in Pl. X, Figs. 8 and 9 have been figured by Renault as examples of *C. Grand'Euryi* (16, Pl. LXII, Figs. 1 and 2) Their existence is of itself sufficient to make the validity of a specific separation of *C. Decaisnei* from *C. Grand'Euryi* doubtful, since they show that in the latter there were sometimes but fourteen sporangiophores and probably only about twenty-eight bracts in the whorl. Renault seems greatly to

TABLE I.

Date of Publication.	Bracts.		Sporangiophores.		Axial Bundles.	
	C. Grand'-E	C. Decaisnei.	C. Grand'-Euryi.	C. Decaisnei.	C. Grand'-Euryi.	C. Decaisnei.
1876, 1878, and 1882	36	24	18	12	{ 18 main 36 small	12 main 24 small
1888 and 1890	—	24	--	12	variable	{ 6 main 12 small
1896 and 1898	36	24	18	12	{ 18 main 18 small	12 main 12 small

TABLE II.

Section seen in Pls. IX and X	Bracts.	Sporangiophores.	Axial Bundles.
Fig. 8	*circa* 28 (e)	14	6 main, 12 small
Fig. 9	*circa* 34–6 (e)	16–18 (e)	6–7 main, 12–14 small (e)
Fig. 5	36 (e)	—	10–11 main, 14 small (e)
Fig. 7	26–8 (e)	—	7 main, 12 small
Unfigured (2)	26–8 (e)	—	7 main, 12 small
Fig. 6	*circa* 30–2 (e)	—	8–9 main, 16 small
Fig. 10	24 (e)	12	6 main, 12 small
Fig. 11	—	—	6 main, 12 small
Unfigured	—	--	6 main, 12 small (e)

have overestimated the number of bundles in the axis of his *Arthropityo-stachys Grand'Euryi*. He gave it variously as 54 (18 main, 36 small) and as 36 (18 main and 18 small), whereas there were in the two sections he figured respectively 18 (6 main and 12 small) and about 20 (7 main and not more than 14 small). In point of fact 18 (6 main and 12 small) is exactly the *lowest* figure quoted by Renault for *C. Decaisnei*, while the figure 20 comes nearer to the lowest than to the higher figures (36 and 24) quoted for that species, and is, of course, much below any figure quoted for *C. Grand'Euryi*! If we turn from the internodal to the nodal sections the position is equally bewildering. A portion of the section shown in Fig. 5 was figured by Renault as *C. Grand'Euryi*, and there is reason to believe that at this node there were about 36 bracts. Even here, however, there do not seem to have been more than 10 or 11 main bundles and 14 smaller ones. In the section shown in Pl. IX, Fig. 6, where there were probably about 30 bracts, 8 or 9 main and 16 smaller bundles, we have a section which is not

only not referable either to *C. Grand'Euryi* or *C. Decaisnei,* but is actually intermediate between them in regard to both the distinctive characters which can be observed in it. The section of which Pl. IX, Fig. 7 is a photograph and the two sections in the Renault collection apparently cut just above it (see pp. 334–5) seem to be examples of a cone, or possibly of cones, rather nearer to Renault's *C. Decaisnei* than to his *C. Grand'Euryi,* though the cone (or cones) from which they come seems to have possessed rather more bracts in a whorl than Renault's *C. Decaisnei.* The number of bundles too, though not as great as the highest number quoted by Renault for the latter species. is, nevertheless, rather greater than in the last three sections of Table II, which undoubtedly belong to this type. The general similarity of the sections shown in Pl. IX. Fig. 7 and Pl. X, Fig. 11 is very striking. Apart from the considerably greater size of the stele in Fig. 7, the only real difference lies in the fact that the section shown in this figure passes so close to the node that on one side a certain amount of secondary xylem can be seen, whereas the section represented by Fig. 11 is cut farther from a node and possesses no secondary xylem.

These facts justify the conclusion that Renault's *Arthropityostachys Grand'Euryi* and his *A. Decaisnei* belong to the same species, in the sense in which the word species is generally understood in Fossil Botany, the larger specimens or larger portions of cones corresponding to *C.* (Renault's *Arthropityostachys*) *Grand'Euryi* and the smaller specimens or smaller portions of specimens to *C.* (his *A.*) *Decaisnei.* Since in Renault's first description the name *Bruckmannia Grand'Euryi* precedes *B. Decaisnei* we may retain the specific name of *Grand'Euryi.* It may, perhaps, be convenient to use the expression *forma Decaisnei* for smaller specimens with a regular type of stele. Typically, *forma Decaisnei* possessed 6 main and 12 smaller axial bundles, 12 sporangiophores and 24 bracts, though variations in the number of appendages and bundles doubtless occurred also in the smaller type of cone.

IX. Diagnosis of *Calamostachys Grand'Euryi*, Ren., Emend.

Calamostachys Grand'Euryi, Ren., according to this enlarged conception, may be described as follows :

The axis bore successively equidistant fertile and sterile whorls, the members of the latter whorls being approximately twice as numerous as those of the former. The number of sporangiophores in a whorl varied at least from 12 to 18, that of the bracts at least from 24 to 36. Apart from disturbances due to these variations in number, bracts and sporangiophores seem to have been superposed respectively to members of the similar whorls above and below. There was, however, no constant relation either of superposition or of alternation between the sporangiophores and bracts, or pairs of bracts, of the whorl

below, the two kinds of organ being apparently inserted on different systems on the axis.   The highly characteristic bracts were basally united into a disc, from the lower surface of which a series of radiating parenchymatous plates, equal in number to the sporangiophores, extended downwards to the level of, or a little below, these organs.   The sporangiophores were peltate and bore four sporangia.   All the spores observed are of one kind and, as a relatively large number of specimens showing sporangia from various levels in the cone are known, there is a strong presumption that the cone was homoporous.   The axis contained two kinds of vascular bundles, arranged round a pith which became fistular.   Some of the bundles were larger and deeper seated than the others, and these were usually approximately, though only approximately, equidistant.   The number of bundles in the axis varied considerably in different cones and at different levels, but we have no direct evidence that there were ever fewer than 6 or more than 11 (or perhaps 12) main bundles, while the number of the smaller bundles varied, in the cases observed, from 12 to 16.   Main and small bundles alike developed a considerable amount of secondary xylem in the neighbourhood of the nodes, though elsewhere all the xylem seems to have been primary and centrifugal.   Usually the bundles were continuous through the nodes, though occasional anastomosis occurred at or near this level.   Usually the small bundles gave off one and the main bundles two bract traces, but occasionally a small bundle gave off two such traces.   The smaller bundles ran opposite to the sporangiophores and gave off traces to them, but when the latter were more numerous than the former the main bundle nearest to the supernumerary sporangiophore gave off a trace to it.   Most—sometimes all—of the main bundles, however, lay definitely between certain of the sporangiophores and took no part in the emission of traces to these organs. In some cases at least, the traces of the sporangiophores arose somewhat above the latter organs and were deflected downwards in their course through the cortex.   It would seem, however, that the amount of this downward deflexion was sometimes slight, and there may have been levels at which it did not exist.

X.   The Affinities of *C. Grand'Euryi*.

Appendages extending downwards from the bracts seem to be known only in one other species of *Calamostachys*, in *C. mira*, Weiss.[1]   This cone is certainly not specifically identical with *C. Grand'Euryi*.   It was a smaller fructification with internodes but little more than 4 mm. in length and with not more than eighteen short, free bracts in a whorl (21, pp. 43-5, Pl. III, Fig. 1).   It is only known in the form of impressions, and in our

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[1] *Huttonia spicata*, Stbg., has been shown by Stur to be a *Palaeostachya*: see Jongmans, 8, p. 354.

ignorance of its anatomy we cannot say how close is its affinity to *C. Grand'-Euryi*. *C. Grand'Euryi* appears to be sharply differentiated from the other petrified Calamarian cones known to us by the possession of two kinds of axial bundles, large and small. The main bundles apparently correspond to the ordinary bundles of the axes of Calamarian cones, but the smaller bundles appear to be peculiar to *C. Grand'Euryi*. The anatomy of *Calamostachys Zeilleri* and *C. magnae-crucis* may, perhaps, afford the clue to the origin of these smaller bundles. It will be remembered that in these two species the traces of sporangiophores arose near the level of the node below and ascended within the axis through at least half the internode before passing out into the sporangiophores. If, instead of passing out, these strands had continued their course through the axis, each merely giving off a branch to a sporangiophore, we should get a series of small strands comparable to those of *C. Grand'Euryi*. In the latter species there are typically two small strands between and slightly outside the main bundles. Thus in size, number, and position sporangiophore traces and small bundles resembled one another. Again, according to Renault, the traces in *C. Zeilleri* ascended above and were deflected into the sporangiophores. The fact that the analogy with the recent genus *Equisetum* suggests that this deflexion (observed also in some specimens of *C. Grand'-Euryi*) varied at different levels in the cone and in cones of different ages, and that it may have been absent from younger and more apical regions, does not invalidate the conclusion that a certain tendency for the traces of the sporangiophores to persist into the upper half of the internode existed in *C. Zeilleri*. A further development of this tendency, leading eventually to the persistence of these strands through the internodes and nodes, and consequently to their conversion from mere traces into axial bundles, may well have been correlated to an increase in the number of bracts, both actually and proportionately, to the bundles. The absence of any specimens of Renault's *C. Zeilleri*, combined with his curious tendency greatly to exaggerate the number of the axial bundles, makes it unsafe to carry the comparison with this species farther. But if we pursue the comparison of *C. magnae-crucis* with *C. Grand'Euryi* we see that in the former the bracts were three times as numerous as the bundles, and that the proportion they bore to the sporangiophores was about as three to two. Now, if the proportion of bracts increased until they were twice as numerous as the sporangiophores, as seems to have been the case in *C. Grand'Euryi*, then each bundle would have had to give off traces to four bracts. But the departure of four traces, destined to equidistant bracts, from each bundle would produce a congestion at the point of origin of the traces and necessitate a considerable divergence in the course of some of the latter. It would seem, therefore, that *pari passu* with the increase of the bracts there was evolved a persistence of the former traces through internode and nodes and an

assumption by them of the vascular supply of the nearest bract.   Thus the number of bract traces supplied to each main bundle was reduced to two, and as the bundles, small and large alike, were equidistant, this rearrangement ensured a more even insertion of bract traces on the stele and lessened the length of their course through the cortex.   The former traces, now axial bundles, not only themselves gave off traces, but also produced secondary xylem at the nodes.   Nevertheless, they retained their peripheral position and, in spite of their smallness, the function of supplying traces to the sporangiophores.   It was only when the latter were more numerous than the small bundles that the main bundles supplied traces to the additional sporangiophores.

No doubt the phylogenetic origin of the smaller bundles was complicated by countless interactions, such as those resulting from variations in number of the appendages.   It is not, of course, contended that *C. magnae-crucis* or *C. Zeilleri* represents an ancestor of *C. Grand'Euryi*.   Indeed, the superposition of the bracts in the last species seems to be a more primitive character than their alternation in the other two species ; and, in the alternation of the bundles at the nodes, *C. magnae-crucis* shows a higher degree of specialization than *C. Grand'Euryi*.   It is only suggested that the unusual vertical extent of the course of the traces of the sporangiophores in *C. Zeilleri* and in *C. magnae-crucis* may supply the clue to the evolutionary origin of the smaller axial bundles in *C. Grand'Euryi*.   Even for this the evidence is insufficient.   In particular the discovery of sections through the bractigerous node of *C. magnae-crucis* and the re-discovery of Renault's sections of *C. Zeilleri* are desirable, if the suggested phylogeny of the small bundles is to be confirmed.

PART III.   ON THE PROBABLE STRUCTURAL FORM OF *CALAMOSTACHYS CALATHIFERA* (WEISS).

Among the sections of the Renault collection at Paris is a well-preserved radial longitudinal section through a species of *Calamostachys*.   This section seems never to have been described or figured by Renault, and is shown in Pl. XI, Fig. 18.   Pl. XI, Fig. 19 represents a portion of the same section on a larger scale.   The complete section is about 1·4 cm. long and includes four nodes.   As the width of the axis and the length of the internodes hardly vary, the section probably represents a fragment of a considerably larger cone. The internodes are about 4 mm. long and the axis nearly 2 mm. wide in this region, but attaining about 2·75 mm. at the nodes.   A wide central cavity, hardly, if at all, narrowed at the nodes, occupies about four-fifths of the axis.   At the nodes there seems to have been some secondary xylem, though elsewhere the woody cylinder is so narrow that it is probable that

only primary xylem was present. The section misses the actual insertion of the bracts, but shows, at the third node, two of them cut nearly radially. They appear to be obliquely ascending from their very bases, and their very acute apices only extend upwards to a little beyond the insertion of the sporangiophores. The latter are inserted midway between the whorls of bracts, and, although the section does not pass radially through the sporangiophores, the disposition of the sporangia suggests that the former were arranged in superposed whorls. The section shows small portions of the stalks of several sporangiophores, and in the case of the two lowest stalks on the reader's right it can be seen that their traces arise markedly above the sporangiophores, more than three-quarters of the way up the internode, and are deflected downwards in the cortex for at least 1 mm. At least in the proximal part of the stalk, which alone is preserved, the trace runs nearer to the upper surface, with reference to the whole cone. The sporangiophores were short, and though the sporangia are well preserved there is no indication of any peltate expansion beyond them. Two only of the sporangia of each sporangiophore are seen in the section, though the latter organs were presumably quadrisporangiate. The sporangia are broadly ovoid structures, their radial depth (under 2 mm.) being but little greater than their height. The spores are small, very numerous, and all of the same kind.

Only one other slide, which was found lying next to this section, seems (to judge from the appearance of the matrix) to have been cut from the same cone. Unfortunately it shows only structureless fragments and there seem to be no other sections which can well be attributed to the same species. We do not, therefore, possess any certain knowledge of the number of bracts and sporangiophores in a whorl, or of bundles in the axis, or of the other characters observable only in transverse sections.

In the absence of any transverse sections it becomes increasingly important to correlate the petrifaction seen in Pl. XI, Figs. 18 and 19 with impressions of Calamarian cones. The cone which it seems most closely to resemble is that described by Weiss, first as *Stachannularia*, later as *Calamostachys calathifera* (21, p. 27, Pl. III. Fig. 3 ; 22. p. 178), a type of cone which has been found by Sterzel in connexion with leafy branches known as *Annularia sphenophylloides*, Zenker (20, pp. 685-90, Pl. XXVIII). This cone seems to have borne at each node about ten to twelve short bracts ending in a sharp point. Weiss says that there were eighteen to twenty bracts at a node, because, although twelve of the thirteen nodes seen in the single fragment figured by him show only about five bracts in the half-whorl, the lowest node shows nine or ten bracts. He, therefore, thought that above the lowest node one side of the cone had been broken off. His figure, however, suggests that at the lowest node the bracts at the back part of the whorl have been flattened out, so as to show in the impression. This appears also to be Dr. Jongmans's view, since

he gives the number of bracts in a whorl as eight to twelve (Jongmans, 8, p. 295) ; it is, however, of course, not impossible that in the lower part of the cone the bracts were rather more numerous.   In Sterzel's more numerous specimens there seem to have been usually five, more rarely four or six, bracts in the half-whorl visible.   This slight variation is probably due to the fact that, as may be seen in both Weiss's and Sterzel's figures, the bracts of one whorl alternate with those of the next.   Weiss mistook the narrow bracts for their midribs.   Both authors speak of the bracts as about as long as the internodes, Sterzel also saying that they are almost as long as these.   These statements may be regarded as accurate, but it must be remembered that owing to their curvature round the base of the sporangia
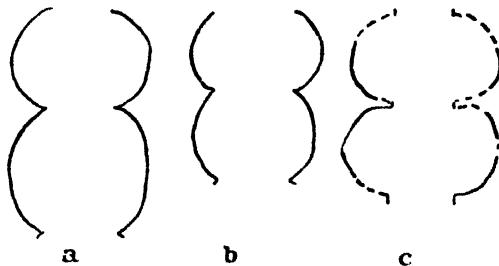
TEXT-FIG. 3. Outline of various cones attributed to *C. calathifera*, Weiss. *a* = internodes b and c of Sterzel's Fig. 3 A ; *b* = two lower internodes of Sterzel's Fig. 2 ; *c* = second and third internodes of the cone shown in Pl XI, Figs. 18 and 19 ; the dotted lines mark the places where the bracts or sporangia are missing or slightly displaced.   × 6.

the bracts do not reach to the level of the next node.   This is in agreement with Sterzel's drawings, though not with Weiss's less distinct figure.   Sterzel also says that the sporangiophores probably alternated with the bracts. This statement has been copied by later authors (15, p. 47 ; 16, p. 71), in some cases with the added deduction that the sporangiophores were probably equal in number to the bracts (8, p. 295).   Sterzel's figures (excluding 4 A, which is a mere reconstruction) strongly suggest that while the bracts alternated the sporangiophores were superposed, a common arrangement in *Calamostachys*.   This is best seen in Sterzel's larger scale Fig. 4, reproduced by Dr. Jongmans (8, p. 295, Fig. 247).   This figure also suggests that the sporangiophores were not as numerous as the bracts, probably not more than six in the whorls figured.   The impressions seem to have been of cones 5½–7 mm. in diameter, with an axis about 2 to 3 mm. wide at the nodes.   The internodes were 4 to 5 mm. long.

Now, the similarities between these impressions and the cone seen in Pl. XI, Figs. 18 and 19 are very striking.   The general dimensions are similar. The petrifaction, being a radial longitudinal section, does not show the number or the width of the bracts ; but the fact that it does not pass through the insertion of any bracts and shows relatively few detached

portions of them makes it probable that the latter were narrow and not numerous. The unusual shortness of the bracts, their pointed apices, and the way they are directed obliquely upwards almost from their very bases are characters common to the impressions and the petrified material. Even more striking is the similarity of outline of the petrified cone to the impressions. The cones preserved as impressions have a peculiar appearance; they seem to consist of a series of superposed almost spherical bodies, corresponding to the internodal region covered by the sporangia. This peculiar conformation reproduces very closely the form which the cone shown in Pl. XI, Fig. 18 would have had in an impression. This is well seen in Text-fig. 3, in which *a* and *b* represent in outline internodes of Sterzel's figures, while *c* represents the outline of the second and third internodes of the petrified fragment. These outlines have been drawn to the same scale ; the dotted lines in *c* represent regions over which bracts or sporangiophores are missing, or have been slightly displaced.[1] Again, Sterzel describes the sporangia in his impressions as probably ovoid, with the narrower end directed to the exterior, a description which applies equally well to the sporangia seen in Pl. XI, Figs. 18 and 19. Finally, in the impressions as in the petrifactions, the sporangiophores seem not to have possessed any marked distal expansions. That this was so also in the impressions is shown by Sterzel's observation that practically the whole area between the bracts is occupied by a delicate sculpturing analogous to that of the sporangial wall in an allied species, *Calamostachys tuberculata*, the cone of *Annularia stellata*, Schlot. (= *A. longifolia*, Bgnt.).

Renault has recorded the occurrence of *Annularia sphenophylloides*, Zenker, from several localities near Autun (16, p. 72), the region from which most of the material for his slides was collected. In view of this fact and of the similarities between the petrified cone seen in Pl. XI. Figs. 18 and 19, and the impressions of *Calamostachys calathifera*, Weiss, known to be the cone of *Annularia sphenophylloides*, it is probable that the cone represented in Figs. 18 and 19 is the structural form of *C. calathifera* and the petrified cone of *Annularia sphenophylloides*.[2]

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[1] Text-fig. 3 A has been enlarged from Sterzel's natural-sized figure, because in his figure on a larger scale this author has drawn the internodes as slightly longer and more slender.

[2] A very different type of cone, *Volkmannia sessilis* or *pseudo-sessilis*, with sporangiophores inserted just below the bracts, was regarded by Grand'Eury as the cone of *A. sphenophylloides* (6, p. 43), while Schenk has ascribed similar cones from China to *A. brevifolia*, Bgnt., which is one of the synonyms of *A. sphenophylloides* (Schenk, 1888, p. 233). But these attributions are now no longer accepted (9, p. 37 ; 10, p. 711-12).

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EXPLANATION OF PLATES IX–XII.

Illustrating Miss Browne's paper on Cones of the Calamostachys Type in the Renault and Roche Collections.

PLATE IX.

Figs 1–7. *Calamostachys magnae-crucis* and *Grand'Euryi.*

Fig. 1. Transverse section through a cone of *Calamostachys magnae-crucis*, n. sp., showing the bases of some of the sporangiophores and portions of thirty-two sporangia belonging to the lower series. Outside these may be seen, on one side of the section, a certain number of sterile bracts cut transversely. Note the eight main lacunae or bundles, situated between every two sporangiophores, and, on one side of the section, the dark patches or traces entering the sporangiophores on the side remote from the neighbouring large lacuna. × 12.

Fig. 2. Transverse section (from the Roche collection) through a cone of *C. magnae-crucis*, showing portions of the sporangiophores, sporangia, and bracts. The sporangia belong to the upper series. × 12.

Fig. 3. Tangential longitudinal section through part of a cone of *C. magnae-crucis*. Note the close similarity of this section to that figured by Renault as *Calamodendrostachys Zeilleri* in Fig. 3 of Pl. LX of the Atlas of 1893 (16). Renault's figure probably also represents *Calamostachys magnae-crucis.* × 5.

Fig. 4. Tangential longitudinal section through part of a cone of *C. magnae-crucis*. To judge from the coherence of the bracts in the lowest whorl this section is more deeply seated than that shown in the previous figure. × 8.

Fig. 5. Transverse section of part of the axis of a cone of *Calamostachys Grand'Euryi* taken at the node. Note the large and small bundles and, on one side of the section, the traces departing to the bracts. Towards the periphery of the photograph is a transverse section of a large, detached bract of the type characteristic of this species. Renault's Fig. 6 of Pl. LXII of the Atlas of 1893 (16) represents a portion of this section. × 5.

Fig. 6. Transverse section of a cone of *C. Grand'Euryi* cut near the level of the node, but slightly above the departure of the traces. Note the large and small bundles and the secondary xylem. The dark middle portions of the coherent bracts represent the sclerenchymatous strand lying above the bundle. × 12.

Fig. 7. Transverse section from near the node of a smaller cone of the same species. On one side of the stele there is still a certain amount of secondary xylem, but on the other, higher side there are hardly any secondary elements. × 12.

PLATE X.

Figs. 8–13. *Calamostachys Grand'Euryi.*

Fig. 8. Transverse section of a cone of *C. Grand'Euryi* passing through the sporangiophores. Note the canals of varying sizes and situated at somewhat different depths. Renault's Fig. 1 of Pl. LXII of the Atlas of 1893 represents half this section considerably restored and idealized. × 12.

Fig. 9. Transverse section of a cone of *C. Grand'Euryi* taken slightly above the level of the sporangiophores. Note the radiating plates of cellular tissue extending downwards from certain of the bracts to the sporangiophores. Between these lie the sections of the upper series of sporangia. Note also the larger, deeper-seated and the smaller, more peripheral lacunae. Outside the sporangiophores are the remains of numerous bracts, some probably *in situ*, but most of them detached and cut in very varied planes. This section represents the original of Renault's diagrammatic and restored Fig. 2 of Pl. LXII of the Atlas of 1893 (16). × 12.

Fig. 10. Transverse section through the cone of *C. Grand'Euryi, forma Decaisnei*, at the level of insertion of the sporangiophores. Note the larger, deeper-seated and the smaller, more peripheral canals ; the sporangia ; the remains of the cellular plates, and outside these the bracts, of which the sclerenchymatous strand alone is preserved. This section has been figured by Renault under the name of *C. Decaisnei* (12, Pl. IV, Fig. 3). × 12.

Fig. 11. Transverse section of a cone of *C. Grand'Euryi, forma Decaisnei,* a little way above the insertion of the bracts. Note the regular disposition of the large and small canals and the general similarity of the section to the larger or typical form of *C. Grand'Euryi* shown in Fig. 7. × 12.

Fig. 12. Longitudinal section of part of a cone of *C. Grand'Euryi.* Note the detached fragments of the cellular plates and of the characteristic bracts. × 6.

Fig. 13. Upper part, more highly magnified, of the section shown in Fig. 12. The departure of a bract trace can be seen at the upper node on the reader's right. × 12.

PLATE XI.

Figs. 14–19. *C. Grand'Euryi* and *C. calathifera.*

Fig. 14. The lower part of the section represented in Fig. 12 on a larger scale, showing the irregular anastomosis of the bundles at the node. × 12.

Fig. 15. Radial longitudinal section of part of a cone of *C. Grand'Euryi, forma Decaisnei.* Note that where the section is truly longitudinal the bundles, represented by their lacunae, are continuous through the node. Note, too, the cellular plates extending upwards from the sporangiophores to certain of the bracts of the whorl above. Near the upper edge of the photograph may be seen the characteristic transverse sections of detached bracts. × 5.

Fig. 16. Portion of the same section much more highly magnified. Note the downward deflexion of the sporangiophore trace on the reader's right, near the upper end of the photograph, and the radial section of the cellular plate extending upwards from the sporangiophore supplied by this trace; also the continuity of the canals through the node and, at the periphery, the remains of the upturned bracts of the whorl below. × 20.

Fig. 17. Radial longitudinal section through one side of the axis of the cone of *C. Grand'Euryi, forma Decaisnei.* Note the continuity of the bundle through the node and the marked deflexion of the traces of the two sporangiophores in their course through the cortex; also the numerous detached sterile bracts. × 12.

Fig. 18. Radial longitudinal section through part of a petrified cone of *Calamostachys calathifera,* Weiss. Note the superposition of the sporangiophores, devoid of any obvious peltate expansions; the globular sporangia and the short bracts, ascending from their very bases. × 6.

Fig. 19. Portion of the last section more highly magnified. Note on the reader's right the marked downward deflexion of the traces of the two sporangiophores traversed by the section. × 12.
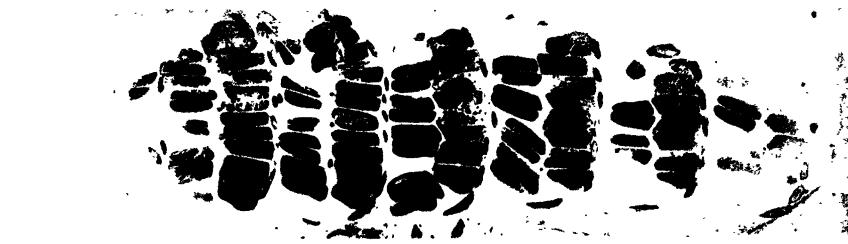
BROWNE— CONES OF CALAMOSTACHYS TYPE.

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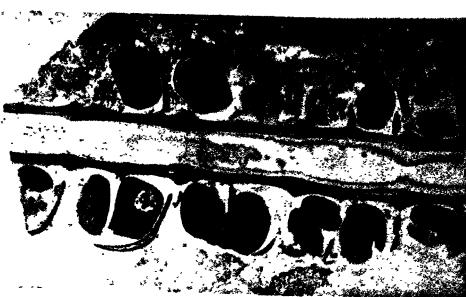
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BROWNE—CONES OF CALAMOSTACHYS TYPE.

Polarity Phenomena in Seakale Roots.

BY

W. NEILSON JONES.

With nine Figures in the Text.

THE shoots and roots of many plant species, cut into pieces and allowed to regenerate, exhibit a more or less well-marked polarity. The end towards the shoot apex (the 'shoot-apex end') produces shoots, while the other end (the 'root-apex end') produces roots, although no morphological distinction between the respective ends may be apparent. The pieces of tissue behave as though they possessed an innate polarity determining the production of roots and shoots respectively.

Species differ from one another in respect to the intensity with which this polarity is exhibited, especially when the environment is such as to act in opposition. Thus, a piece of stem of *Aloe frutescens*, which produces buds from the stem-apex end and roots from the root-apex end when allowed to regenerate in a horizontal position under uniform conditions, will produce roots from the stem-apex end and vice versa if placed with the stem end downwards during regeneration : i. e. the action of gravity may overpower the innate polarity and cause a production of roots from the *lower* end. On the contrary, *Rosa indica* has a polarity so strongly marked that shoots are produced from the shoot-apex end of the cutting even when this is downwards.

Massart (17) holds that, broadly speaking, plants can be divided into four groups in accordance with the regeneration behaviour of cuttings when planted upside-down, namely : (1) strongly marked stem and root polarity; (2) strong stem polarity but weak root polarity ; (3) strong root polarity but weak stem polarity ; (4) weak stem and root polarity. On this view, *Rosa indica* belongs to group (1), *Aloe frutescens* to group (4). This grouping emphasizes the fact that the nature of the polarity affecting shoot production may be quite unlike and independent of that affecting root production. It seems unlikely, however, that all cases will lend themselves to so rigid a classification.

Now, the production of lateral or adventitious roots or shoots on the part of isolated portions of a plant is a complex phenomenon involving several steps: (1) the origin of a meristem; (2) the further growth and development of such meristem; (3) the differentiation during development into either a root or stem structure. Any investigation into regeneration phenomena must take account not only of the existence of these stages of the process, but of the fact that each of them may be controlled by a different set of factors. Thus, inherent polarity may express itself (*a*) in regard to the positions in which meristems arise; (*b*) which of several meristems develop the more strongly; or (*c*) whether the meristem develops into a stem or root. For polarity to manifest itself in any of these ways the formal conditions required for normal growth must, of course, be provided.

A considerable amount of experimental data has been collected touching upon regeneration and polarity from all three of these aspects. A brief review of some of the more significant contributions to the subject is given below.

(*a*) *Factors which predispose a tissue to become meristematic.*

When 'cuttings' are allowed to regenerate, the development of a callus tissue is usually, though not invariably, the first product of meristematic activity. This callus is derived principally from the cambium in the neighbourhood of the wound, but living cells in other positions may take part in its production. Regions of more intensive growth arise within the callus leading to the formation of cork, shoots, or roots. The latter often arise from the pericycle without the intervention of a callus tissue.

The formation of meristems in connexion with the healing of wound surfaces has been studied extensively. In a recent paper, Priestley and Woffenden (20) have reviewed the literature of this subject and added a number of new experimental observations. The main conclusions of these authors are as follows:

(1) Meristem formation occurs below a surface that has become impervious to the passage of water, i. e. either blocked naturally by the formation of cork or by some artificial means.

(2) Meristem formation depends upon the existence of a sap pressure leading to the accumulation of sap at the blocked surface, i. e. the stimulus to cell-division is dependent upon the accumulation of substances derived from the xylem, rather than from the phloem as believed by Haberlandt.

In another communication by Priestley and Ewing (21) these views are elaborated and supplemented. It is suggested, for example, that the endogenous development of lateral roots follows from the hold up of sap at the endodermal boundary, while lateral shoots develop exogenously

because the endodermis is poorly developed in shoots and 'block' of the sap occurs at the epidermal, instead of at the endodermal, layer. Etiolation phenomena, &c., are discussed from the same point of view.

Without entering further into details, it follows on this view that 'polarity', resulting in the formation of meristems in special regions, may be the result of a structure favouring the flow of material in a particular way, and hence, in the words of the authors, 'an inevitable outcome of the reaction between the earlier developmental stages and its environment'.

Meristems may also arise apart from wounding, as in the formation of adventitious buds of *Begonia, Bryophyllum, Aspidium*, the climbing roots of Ivy, &c., the ' peg' of *Cucurbita* seedlings, &c. Further investigation is required before it is possible to say in any given case whether the position in which the meristem appears is determined by anatomical structure controlling the direction of flow of material or by an innate 'polarity' of the protoplasm.

(b) Relative vigour of the development of several meristems.

The major portion of the work of investigation on the nature of polarity in plants relates to this aspect. It is not possible here to do more than indicate under various heads the kind of work that has been done ; for further details the various papers mentioned in the bibliography may be consulted.

One of the cases falling within this category relates to the order of development of the axillary buds on the shoot. In general, the terminal bud is the first to grow out, and develops most vigorously, the lateral buds developing with less and less vigour as the base of the shoot is approached. A bud which would normally remain dormant, however, may be often induced to develop by special treatment, e. g. removal of the terminal bud or terminal portion of the shoot. The pruning operations practised by horticulturists are based upon this principle.

A large amount of experimental work has been carried out in the attempt to obtain an insight into the causes which induce one bud to develop rather than another, and so to obtain control over the manner of development.

Among the simpler of such investigations are those in which shoots are allowed to develop when orientated in various ways. As mentioned above, such experiments show that species differ among themselves in regard to the effect of gravity on the order of development of lateral buds. In some species the order is the same however the shoot is placed, the basal buds showing least vigour of development ; in other species reversal of position induces the basal buds—now uppermost—to develop most vigorously. In short, while in some species the internal polarity controlling order of development seems to be fixed, in other species gravity

appears to be able to overpower it. The difference is not absolute and various gradations between the extremes of behaviour occur.

Stimulus to development may be given to a bud by removal of other buds. This is shown in the operation of pruning as already noted, and is even more markedly exhibited by the non-development of buds arising in the axils of cotyledons of seedlings, e. g. *Phaseolus*, so long as the terminal bud of the epicotyl is present. These cotyledonary buds develop, however, on removal of the terminal bud or suppression of its activity by surrounding it with an atmosphere of hydrogen, chloroform, &c. Wounding of the seedling in regions nearer the cotyledons than the epicotyl, local variation in the environment as regards moisture, &c., are without effect—development of the cotyledonary buds appearing to be definitely associated with suppression of the metabolic activity of the terminal bud of the shoot (16).

Two explanations have been offered of this influence of the terminal bud upon development : one of these assumes a translocation of material, either by removal of formative substances from the neighbourhood of the cotyledons or by accumulation of inhibitory substances there ; the other involves transmission of a stimulus. The experimental data available favour, on the whole, the latter supposition. Thus, it has been found that the terminal bud can be isolated, in regard to the effect in question, by subjecting the part of the stem between it and the cotyledons to the action of an anaesthetic or to a low temperature (2° C.). Such 'physiological isolation', while not affecting to an appreciable extent the transmission of water, &c. (or presumably that of 'formative' or 'inhibitory' substances, if such exist), induces development of the cotyledonary buds in the same way as does removal of the epicotyl, so long as the condition responsible for the 'isolation' is maintained (7).

Similar phenomena are associated with the production of adventitious roots by stems. Thus in Willow, the origin and development of adventitious roots from stems is associated with the presence of numerous dormant meristems in the pericycle. The factors controlling the order of development of these root meristems are apparently similar in kind to those effective in the case of lateral buds in the same plant, but they operate in a converse manner. Consequently, on an isolated shoot it is the basal roots which tend to develop most strongly. As in the case of lateral shoots, the effectiveness of gravity in modifying the order of development of adventitious roots varies with species.

Experiments upon *Phaseolus* seedlings have shown that the production of lateral roots from the stem is controlled from the root end of the seedling, just as, in the same plant, the development of lateral shoots is controlled by the activity of the apical bud. Thus, if part of the stem of a seedling is kept moist or under water, adventitious roots only emerge

subsequent to removal of the root system, when they develop from the lower part of the stem. Moreover, if a transverse cut be made in the part of the stem under water, so as to sever one group of the longitudinally running vascular bundles, lateral roots arise close to and above the region of the cut from the side of the stem in which isolation from the root and lower part of the stem has been brought about by cutting through the bundle (16).[1]

From such experiments it appears that the retarding influence of buds on bud formation or development always passes *down* the stem, while the retarding influence of roots on root formation or development always passes *upwards*, and, moreover, passes by way of the vascular bundles.

Though it adds little to an understanding of the causes underlying the facts, Child's conception of a 'metabolic gradient' is valuable in so far as it permits co-ordination of the observed facts (5, 6). Child considers that metabolic activity, as measured by intensity of respiration, susceptibility to poisons or stimuli, &c., varies in different parts of an organ in a definite manner: so that in passing along the organ one passes into regions of increasing metabolic activity or the reverse. In the stem of a plant, for example, the apical meristem is the region of highest metabolic activity, there being a gradient of decreasing activity as one passes away from the apex. Similarly, the meristems of lateral buds are regions of relatively high metabolic activity and form the apices of backwardly extending local metabolic gradients. Hence, the whole shoot system may be conceived as built up of a series of gradients : the main gradient of which the shoot apex is the highest point, and subsidiary gradients of which the lateral apices form the highest points, although all the latter are of relatively lower metabolic activity than the terminal growing point. It is further supposed, as regards development, that regions of higher metabolic activity can undergo independent development and dominate regions of lower activity.

Thus, in the case considered above, the cotyledonary buds being regions of relatively low activity develop only when the influence of the apical bud is removed, either by permanent destruction or by temporary physiological isolation.

One difficulty in the application of this conception to plants is that in these there is apparently the necessity of assuming the existence of two gradients—a stem gradient of which the terminal bud is the highest point, and a root gradient of which the root apex is the highest point. It is, perhaps, a little difficult to conceive of two gradients along the same axis running in opposite directions, unless (1) the gradients are different in kind, or (2) they pass along different tissues. There are obvious difficulties in

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[1] One has to do in this case with the formation of a meristem as well as with its subsequent development.

B b 2

the first supposition and there is litttle evidence for the second. As pointed out above, the root gradient seems to be located in the neighbourhood of the vascular tissue: it is possible that the stem gradient is located in the extra-stelar tissues, but there is at present no evidence for this or for the view that the endodermis may form a bound⬤ between the two gradients.

The facts reviewed in the previous section may be summarized as follows : (1) In a uniform environment the order of development of the different meristem groups of a plant organ appears to be determined by an innate polarity or, to use the conception of Child, by the presence of metabolic gradients in the organ. (2) In some cases anatomical structure, in so far as it determines direction of flow of material in the organ, may be partly responsible for the manner of development. (3) The effects of this innate polarity can be overcome (or the direction of the metabolic gradient reversed) in some cases by the character of the environment, &c., as when the order of development of a series of buds is modified by gravity, by ' physiological isolation '. or by removal of certain buds.

(c) *Differentiation of meristem into shoot or root.*

Included in the problem is the nature of the causes underlying development of a meristem into root or shoot as the case may be. At the present time it is known that such development is governed by the position of the meristem in relation to the rest of the organ, and sufficient is known of these relations for the development into root or shoot in any particular case to be foretold with a fair degree of certainty. It is also known that the effect of these relations can be modified to some extent by environment. We are, however, entirely ignorant as to the deeper-lying causes of the phenomena.

The Present Investigation.

The present work has to do with polarity manifested in the regeneration of roots or shoots from pieces of root ('root-cuttings') of Seakale (*Crambe maritima*). These roots are convenient for experiment for several reasons ; they contain considerable amounts of reserves, they form callus readily, and regenerate both roots and shoots without difficulty if kept in damp moss or cocoa fibre.

The following sections deal with observations on the regeneration of Seakale roots under different conditions :

1. If pieces of Seakale root 5–10 cm. long are allowed to regenerate while lying in a horizontal position, the cut surfaces at both ends develop callus which produces buds from the shoot-apex end and roots from the root-apex end in the course of two or three weeks. This polarity is well marked, and is maintained when the cuttings are placed vertically in the reversed position (root-apex end upwards).

It may be noted that the same group of cells in the middle of a length of root can be made to develop either roots or shoots. Thus, if the root is cut so that the group of cells in question is free on the root-apex side but attached on the stem-apex side, on regeneration the cells will produce *roots* ; if the cut is made slightly nearer the shoot-apex end, this group of cells being free on the shoot-apex side and attached on the root-apex side will regenerate as *shoot buds*.

2. The behaviour of longitudinally split pieces of Seakale root shows that the capacity to form new roots and shoots is not confined to the ends of the cutting. Pieces of the longitudinally split root placed horizontally form roots and shoots from the respective ends as before ; the tissues exposed by the longitudinal cut, especially the cambium, also show activity ; there is a development of buds accompanied by, chlorophyll formation at the shoot-apex end, decreasing in vigour as the root-apex end is approached. The last cm. or so of the lateral callus nearest the root-apex end does not develop chlorophyll and does, in some cases, produce roots. In short, the tendency for production of buds and chlorophyll at the shoot-apex end extends a considerable distance along the lateral callus, becoming weaker as the distance from the shoot-apex increases; on the contrary, the tendency for root production extends only a short way from the root-apex end (Fig. 1, A).

3. If pieces of longitudinally split root are allowed to regenerate in a vertical position, either with the shoot-apex end upwards or in the reverse position, a similar development of the lateral callus takes place. There is, however, a definite shifting upwards of the region of shoot production, i. e. away from the pull of gravity. Thus, the region of bud and chlorophyll production tends to be shorter in the case of cuttings placed the right way up (Fig. 1, B) than in those placed upside-down (Fig. 1. C). The position of root formation is unaffected by alteration of the position of the cuttings.

4. These experiments indicate that gravity influences the localization of bud formation : in view of this fact the effect of subjecting whole pieces of root to centrifugal force was studied. Pieces of root 5–10 cm. long, treated for three days or less on a centrifuge,[1] the shoot-apex end being away from the centre, were subsequently placed in damp moss in a horizontal position. Such cuttings usually produced buds from the root-apex end (Fig. 2), the behaviour being somewhat uncertain when they were centrifugalized for a shorter period than three days. The converse result could not be brought about, i. e. roots could not be induced to arise from the shoot-apex end by centrifugalizing. Controls, in which the shoot-apex end was towards the centre, developed in the usual way.

As a result of experiments on the effect of centrifugal force upon the

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[1] Rate of revolution about 1,000 per min., giving a force of about 7 g. three inches from centre.

order of development of buds in Willow shoots, Küster (10) concluded that the effects of centrifugal force on polarity could be explained by assuming that this force exercises a general retarding effect upon development. He points out that when a piece of tissue is arranged along a radius of the centrifuge, the end farthest from the centre is subjected to a greater force than the end nearest the centre. Therefore, if centrifugal force retards growth in proportion to its magnitude, the end near the centre will show greatest, and the end remote from the centre least, activity of growth. In this way the typical order of development of the lateral buds on a shoot might be reversed, becoming basipetal rather than acropetal.

The behaviour of Seakale roots is not fully explained by such an hypothesis, since there is concerned not merely the difference in vigour of growth at the two ends, but also the fact that a tissue normally producing roots produces shoots under the conditions of the experiment.

5. In developing his hypothesis of metabolic gradients, Child not only suggested the existence of such gradients, but also that the unlike differentiation at the two ends of an organ might depend upon a difference in metabolic activity in these two regions. That a length cut out of the body of a flat worm regenerates a head at one end and a posterior end at the other is due, it is suggested. to the metabolic activity being high at one end and low at the other. On this view, the fact that a short length often regenerates a head at both ends, giving a double-headed individual, is to be expected. In a short length there will be little difference in metabolic activity at the two ends unless the metabolic gradient is very steep ; consequently one end will not obtain dominance over the other, but each will develop independently as a head so far as the available food material allows.

In view of this behaviour of the lower animals, the manner of regeneration of very short lengths of Seakale root is of interest.

If ' slices ' of root 2 mm. or less in thickness be taken, on regeneration they give rise invariably to shoot buds on *both* faces however they are orientated (cf. Figs. 3 and 4).

Considering for the moment the capacity for shoot production only, one may interpret the appearance of buds on the root-apex side of the slice as a result of the tendency for shoot production to spread away from the shoot-apex end of a cutting (p. 365, § 2), which tendency suffers but slight diminution in intensity when the root-apex end is in close proximity to the shoot-apex end as in a thin slice of root.

On the hypothesis of Child, the production of buds from the shoot-apex end of a cutting is due to the high metabolic activity there. The gradient not being steep, however, in a short length of root the metabolic activity at the two ends does not differ appreciably—does not suffice for one end to dominate the activity of the other. Consequently both ends behave as regions of high metabolic activity and give rise to buds.
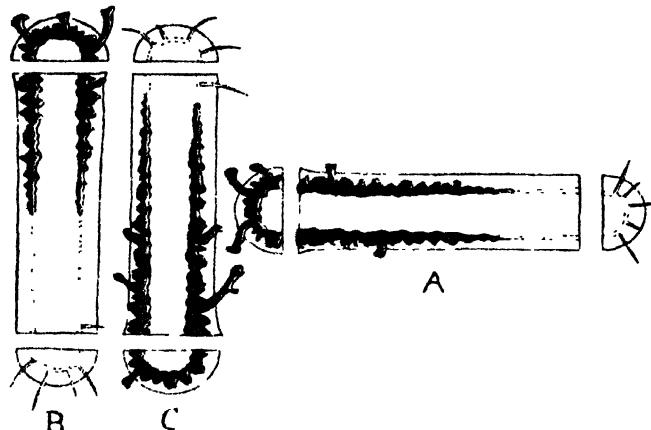
FIG. 1. Diagram to illustrate that the difference in extent of bud and chlorophyll production by longitudinally split Seakale roots is dependent upon their orientation during regeneration. A, root placed horizontally; B, root placed vertically, stem apex upwards; C, root placed vertically, stem apex downwards. In the above, A is shown in plan i.e. exposed surface horizontal), B and C in elevation (i.e. exposed surfaces vertical). A cutting placed horizontally as regards its length, but with the exposed surface in a vertical plane, would regenerate as A, but the upper strip of cambium would develop buds more vigorously as a whole than the lower strip.

FIG. 2. Whole root cutting, centrifugalized for three days and then allowed to regenerate in a horizontal position. Note buds (b) and chlorophyll production from regions normally giving rise to roots only. (Tracing from a photograph.)
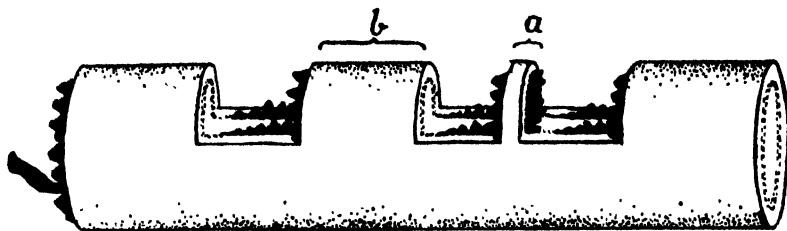
FIG. 3. Diagram to illustrate the formation of buds on both sides of a short length of root (*a*), as compared with bud formation from one end only of a longer length of root (*b*). See Fig. 4.
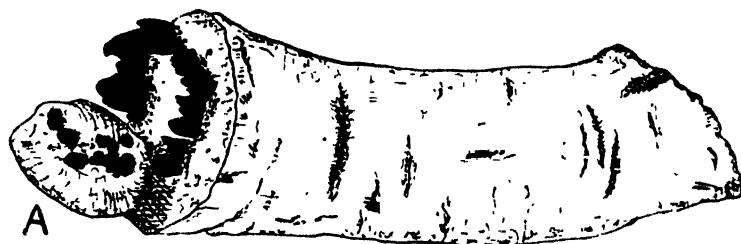
FIG. 4. Production of buds and chlorophyll from the root-end as well as from the shoot-end of a short length ('slice') of root. The thin slice, A, has not been removed completely from the rest of the root and has become shrunken and distorted. The concave, shoot-end face of the slice, which is not visible in the figure, also produced buds. (Tracing from a photograph.

FIG. 6. In the region indicated by the line, the temperature of the cutting was maintained 2° C. higher than elsewhere. Note the buds produced from the root-end of the cutting. (Tracing from a photograph.)

Either of these ways of looking at the matter is satisfactory so far as it goes and so long as one considers the formation of shoots only: they both express the observed facts of behaviour without, however, offering any fundamental explanation of them.

The formation of roots in Seakale root-cuttings appears to be entirely independent of the formation of shoots, and the factors responsible seem to have little tendency to spread from the root-apex end. If root production is due to a high metabolic rate, then either (1) the kind of metabolic change responsible must be different from that in stem production, or (2) the metabolic gradient must operate along different tissues. In either case the gradient must be steeper than is that responsible for stem production. The manner of regeneration in Seakale does not help to a decision between these
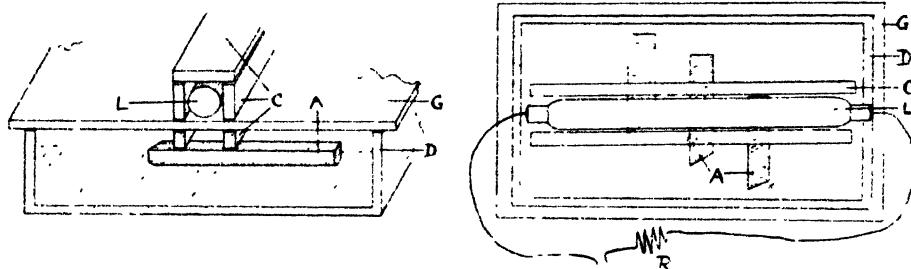
FIG. 5. Diagram of apparatus for warming a limited region of a cutting. A, cuttings; C, cork; D, dish containing peat moss litter; G, glass sheet; L, lamp; R, adjustable resistance.

two alternatives. Certain experiments now to be described do suggest that stem production at least may be associated with a high rate of such metabolic processes as respiration.

6. As already stated, when a longitudinally split root of Seakale regenerates in a horizontal position, development of buds and chlorophyll takes place on the exposed surfaces, especially in the cambium region. This is most vigorous at the stem-apex end and becomes gradually less marked as the distance from this end increases. Experiments were carried out to ascertain whether this manner of development of buds and chlorophyll could be modified by a local increase in the rate of metabolism.

Longitudinally split pieces of root were put to regenerate in a horizontal position, different regions being subjected to slightly higher temperatures. It was found that maintaining a difference of 2° C. was amply sufficient to convert a warmed into a dominant region, as expressed by a vigorous development of shoots, irrespective of its position in relation to the shoot-apex end (Fig. 6). The experimental method adopted is shown in Fig. 5. When whole cuttings were thus treated, shoots could be induced to arise from the root-apex end if a higher temperature was maintained at this end of the cutting. It seems legitimate to conclude from these

experiments that whilst the whole of the cambium has potentiality for bud production, this gains expression chiefly in regions where the cambium is exposed and physiologically most active. Further, while the shoot-apex end is normally the most active region physiologically, as expressed by bud production, it is possible by artificial means to so raise the activity of any other region as to make that region dominant so far as shoot production is concerned.

7. Attention has already been drawn to experiments on Bean seedlings, suggesting that the influence of the root on the production of lateral roots is transmitted along the vascular bundles (p. 363). In Seakale, while no information is available regarding root production, some indication has been obtained as to the tissues concerned in transmission of shoot polarity.

Pieces of root, 10 cm. or more in length, were split into two or more pieces longitudinally; transverse cuts were then made about half-way along their length in such a manner that in some the cortex was severed, in others the pith, and so on (Figs. 7, A, B, and C). It was found that so long as the cambium region was left intact, the piece of root behaved as a single unit with regard to the development of buds and chlorophyll; if the cambium region was severed, however, the root behaved as though it had been cut into so many pieces, in each of which the cambium formed buds and chlorophyll as in a single unit. The exact region has not been determined so precisely that it is possible to say that the cambium alone is concerned, but the cambium and (or) adjacent tissues appear to be those that are effective.

It may be recalled that Barker and Lees (2), in their experiments as to the effect of notching and ringing fruit-trees upon the development of buds, found that a bud made more growth when a notch which removed bark and phloem, and presumably also cambium, was made above it. This would be expected if the severance of the cambium above the bud isolated it from the influence of others nearer the shoot apex.

8. The manner of regeneration from exposed surfaces cut at various angles to the long axis of the root was investigated. Semicircular segments were removed at various places, and observation kept on the behaviour of the exposed surfaces. It was found that the part of the curved surface facing towards the stem-apex end developed buds; on that part of the slope facing towards the root-apex end the development of buds and chlorophyll did not occur, the callus remained colourless and frequently gave rise to roots (Fig. 8, A). The behaviour was the same from whatever part of the cutting the semicircular segment was removed, and the removed segment itself behaved in a similar way (Fig. 8, B). The surfaces exposed by removal of V-shaped pieces of tissue, or cylindrical pieces removed with a cork-borer, behaved in the same way (Figs. 8, C and D). When, however, the two pieces of tissue removed were so close together as to leave but a *thin*

wall of tissue standing, buds were developed from both surfaces of the latter, as might be expected from what has been said regarding the behaviour of thin slices (Fig. 3).

9. Attempts were made to influence development of buds and roots by keeping the ends of the cuttings bathed with solutions having different pH values.

No evidence was obtained that the production of roots or buds was affected, apart from effects that resulted from injury to the tissues in certain cases.

10. In view of the claim of Lund (15) that the polarity of *Fucus* eggs can be determined by the application of an appropriate electric potential, the effect of allowing weak electric currents to pass through the cutting during regeneration was tried in various ways, both with and without removal of the products of electrolysis.

No positive results were obtained. Lund mentions that the limits of difference of potential between which the current is effective in determining polarity in *Fucus* eggs are comparatively restricted, and it may be that lack of success in the case of Seakale root-cuttings was due to failure to hit off the effective difference of potential, although a considerable range of variations was tried.

11. Since the view has been expressed that the opposite responses of shoot and root to geotropic and other stimuli result from the dissimilar electrical charges on particles suspended in the cytoplasm in the two organs, it was thought worth while attempting to determine whether there was any constant difference of potential at the two ends of a cutting.

On the Small hypothesis of 'creaming' (24) it might be imagined, for example, that under normal conditions, when the root is growing in a vertical position, the lighter particles bearing positive (or negative) charges float to the top of the embryonic cells in the growing-point and become partly fixed in that position as the cells mature. When such an organ is inverted these charged particles would remain as before and be responsible for the 'polarity' manifested by the organ by determining a difference of potential in each cell. The effect of gravity or centrifugal force upon the expression of polarity might be attributed to the presence of a certain number of free-moving particles, the varying effects of gravity in different species depending on the relative proportion of free and fixed particles in each case.

No experimental support for this view has been obtained, nor has it been found possible to correlate the behaviour of root-apex end and shoot-apex end with a difference of electrical potential. Attempts to find differences in electrical resistance of the tissues at the opposite ends of cuttings also failed to yield significant or constant results.

SUMMARY.

Attention is drawn to the different ways in which 'polarity' in plants can express itself, and to the nature of the work that has been done to elucidate the phenomena concerned and the hypotheses which have been put forward as a result of this work. Observations concerning regeneration in root-cuttings of Seakale are described, of which the chief may be summarized as follows:

1. Pieces of Seakale root exhibit a well-marked polarity as regards root and shoot regeneration (Fig. 1, A).

2. Expression of this polarity is slightly affected by gravity. The influence of gravity is not strong enough to alter the manner of regeneration at the two ends of a root-cutting, so that the shoot-apex end and root-apex end of a piece of root give rise to buds and roots respectively whichever way up the cutting is planted. The influence of gravity is quite apparent, however, upon the manner of regeneration of the lateral callus developed by longitudinally split pieces of root. In these, the region of the lateral callus developing buds and chlorophyll is relatively concentrated towards the shoot-apex end when the cutting is planted the right way up, and extends much farther towards the root-apex end when the cutting is planted upside-down (Figs. 1, B and C). No effect is apparent, however, upon the position of root formation.

3. Centrifugal force produces similar effects to gravity, but in higher degree. Besides influencing the position of development of buds and chlorophyll on the laterally exposed surface of a longitudinally split piece of root, it is possible to induce buds and chlorophyll to form at the root-apex end of an unsplit root-cutting (Fig. 2). The position of root formation could not be shifted by this means.

4. Short lengths of root, 2 mm. or less in length, always produce shoot buds from *both* ends, but roots from *one* end only (Figs. 3, A, and 4, A).

5. A local rise of temperature tends to make the warmed region of the cutting dominant so far as shoot production is concerned, even though the region in question occupies the root-apex end of the cutting (Fig. 6). A difference of 2° C. is sufficient to produce this effect.

6. The influence of the dominant region of bud formation in controlling the development of other buds appears to be transmitted by tissues in the neighbourhood of the cambium (Figs. 7, A, B, and C).

7. Other methods of overthrowing dominance of the stem-apex end, such as treatment with acid or alkali solutions, electric currents, &c., have all failed.

8. No constant difference in electric potential or of electric resistance
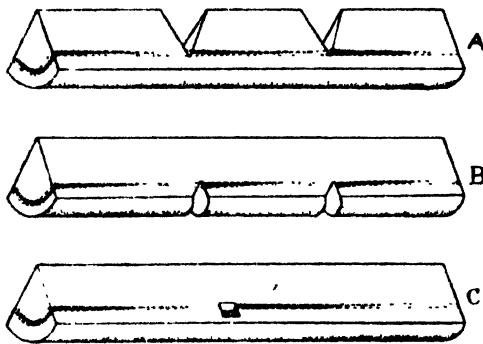
FIG. 7. Diagram to illustrate the effect on the unity of a length of root of severing the cambium while leaving the other tissues intact. A, cambium and tissues inside it cut through; B, cambium and tissues outside it cut through; C, cambium region alone cut through.
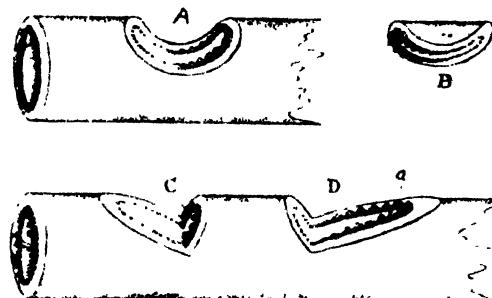
FIG. 8. Diagram to illustrate the manner of regeneration from surfaces exposed by removal of curved segments (A and B) and of wedge-shaped pieces (C and D) from a length of root. In D, note the greater vigour of bud development on the part of the cambium nearest the root-end of the cutting *a*, and compare the manner of development in Fig. 1, A, where the exposed surface is parallel to the length of the root instead of oblique. This is presumably a gravity effect and due to this end of the exposed surface being highest (compare Figs. 1, B and C, which illustrate this tendency).
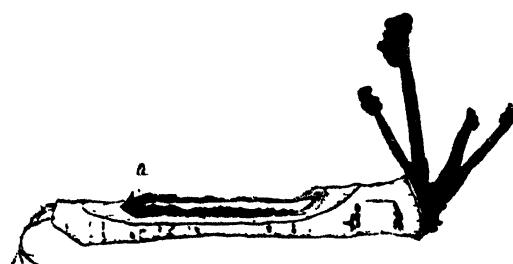
FIG. 9. Similar to Fig. 7, D. The greatest vigour of bud development is at (*a*), towards the root-end of the cutting but the highest point of the slope. (Tracing from a photograph.)

was found to be associated with the root-apex and shoot-apex ends of the cuttings.

9. (4) and (5) indicate that where conditions are otherwise favourable for bud development, such takes place close to regions where processes like respiration are most vigorous. The failure of other potential regions to develop would seem from (6) more likely to be the result of a stimulus rather than due to the transmission of formative or inhibitory substances; this conclusion is in agreement with the views expressed by McCallum, Child, &c., based upon experimental work with other material.

10. The facts recorded are in general accord with Child's conception of 'metabolic gradients', with the exception of (2) and (3), which are somewhat difficult to fit into Child's scheme.

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Studies ...

An Analysis of Factors which ...
forms of Certain Strai...

BY

W. BROWN, M.A., D.Sc.

With eight Figures in the Text.

CONTENTS.

A. INTRODUCTION.

THIS investigation, as has been stated in the general account already published ('Ann. Bot.', xxxviii, p. 379, 1924), was concerned in the first instance with a comparison of six strains of *Fusarium* obtained from diseased apples. The differences between some of these were slight, though none the less quite definite, whereas one or two of them had characters which strongly marked them off from the remainder. When these strains were cultivated simultaneously under identical conditions, they showed

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...and of the much larger number of new ones ,
...time to time by saltation, to carry out a detailed
in... { va ...ty of certain of these strains in response to
...ges o... ... ...ment. In this way only could one arrive at some idea
...o which characters were sufficiently constant to be used in the systematic
treatment of these forms. In reality, however, it was found that there were
very few characters wh ...n were not susceptible of pronounced modification
by cultural treatment, so that the conclusion, as regards the bearing of this
work on the systematic treatment of such forms, is obvious—that any
systematic description which does not relate to strictly defined environ-
mental conditions is valueless. This aspect of the question will be con-
sidered more suitably later on.

The primary object of this work was, however, to study the physiology
and pathology of these forms, and it was considered that the study of their
behaviour as parasites on apple fruit could best be approached by a pre-
liminary study of their behaviour under better known conditions, viz. on
media of known composition. Whether the results obtained regarding the
differential cultural behaviour of these fungi will throw light on their
parasitism remains to be seen. Nevertheless, apart from questions of
pathology, it was felt that a study of these related forms along purely
physiological lines would be of interest in itself.

The problem in question is that of the variability of a fungus under
varied environmental conditions. · That a high degree of variability exists
in many fungi, and in *Fusarium* in particular, is of course common know-
ledge. But the principles underlying this variation and the cultural means
by which one can make a fungus take on this or that growth-form are far
from being equally well known. The great mass of fungal descriptions that
one meets with in the literature relate to the most diversified media—thus.
on potato agar a fungus shows such and such characters, on turnip gelatin
some other characters. The two media cited, quite apart from their
indefinite composition, stand in an unknown relation to each other, and it is
not surprising that the growth appearances of the same fungus on them
show no intelligible relationship with each other. Little information exists
as to why, in the case mentioned, potato agar produces one kind of growth-

form and turnip gelatin another. To the present writer, it appears that information of this kind is urgently needed, not only for one fungus but for many, partly on general physiological grounds and partly with a view to the standardization of methods of technique. Granted that a sufficient body of information of the kind desired were available, one could anticipate much greater efficiency and directness in the cultivation and study of these organisms. The present paper deals with a series of experiments designed to supply such information in the case of a group of *Fusarium* forms.

For the purposes of the present study only a limited number of the strains have been dealt with. A strain, A, which has been longest under observation, and to a less extent another well-defined strain, C_3, have been mostly used in this connexion. All the earlier obtained strains have, however, been tested to a greater or less extent, and though little has been done with some of those which appeared later, a representative set of strains has been examined in fair detail. Thus, while the results to be described below refer chiefly to the strains A and C_3 (and especially to the former), it is known in a general way how far these results are applicable to the others.

As regards methods of experiment little need be said, as the methods employed were in the main those usual in mycological work. The stock cultures were kept in plugged test-tubes. The actual experimental work has been almost entirely carried out with Petri-dish cultures, the inoculum, consisting of spores whenever available, being placed in the centre of the Petri dish, and the development of the colony followed in detail. Any further points of experimental detail will be instanced in the description of particular experiments below.

§ DETERMINATION OF GROWTH RELATIONSHIPS OF VARIOUS STRAINS.

Attention was early directed to the fact that the various strains differed considerably from each other as regards the size of colony produced under a definite set of conditions. Thus, on certain media it was observed that the strain A invariably gave rise to wider-spread colonies than the strain C_3 did under exactly the same conditions. It was decided accordingly to investigate these differences more closely. This method consisted simply in measuring systematically the diameters of the various colonies throughout the whole period of growth. As a method it had the obvious advantages of being simple and also quantitative. In fact colony diameter is one of the very few features of fungal growth which lends itself easily to numerical treatment.

This simple method of study has proved to be of great advantage in the treament of the group of Fusariums under consideration. The incidence of staling, which can by this means be detected, marks a definite physio-

logical reaction, and though the analysis of the problem has not been pushed as yet beyond the merely descriptive stage, it is obvious that a way is open along these lines for the study of the underlying physiological differences which one considers to be responsible for the differential behaviour of the various strains. From the point of view of the work described in the present paper, the advantages of this method of growth study may be enumerated as follows: (1) That certain growth appearances can definitely be correlated with the particular staling intensity shown by different strains or by the same strain under different conditions; (2) that no matter what the final growth-form is, all the strains have over a wide variety of media the same intrinsic rate of growth; and (3) that it is only by reference to the growth-curves that the apparently diverse effects of environmental factors on the growth-form of one strain as compared with another can be reduced to some semblance of order. These points will be brought out in the course of the present paper.

The kind of result obtained may be illustrated by quoting some of the earlier experiments along these lines.

To begin with, the rate of growth on a series of dilutions of the same medium was investigated. The medium first employed was potato agar. A decoction of 200 grm. of potato to the litre was prepared, and the following concentrations of this medium set up: 1/1, 1/4, 1/10, 1/40 (each medium was solidified with 1·5 per cent. agar). Colonies were planted on plates of these media, which were then incubated at 20° C., and the diameters of the resultant colonies determined from time to time. The experiment was carried out in quadruplicate.

In Figs. 1, 2, and 3 the ordinates represent the diameters of the colonies. The various concentrations of the nutrient medium are spaced arbitrarily at equal distances along the *x*-axis. Figs. 1 and 2 relate to strains A and D of the apple organism, Fig. 3 to *Fusarium acuminatum*, a culture of which was obtained from Holland as being, to judge from the published description, somewhat similar to the strains under consideration.

In all the graphs it is seen that the initial rate of growth of the colonies is practically independent of the concentration of the nutrient, the curve representing two days' growth being almost exactly a straight line parallel to the *x*-axis. In the case of *Fusarium acuminatum* (Fig. 3) the curves remain as such right to the end of the experiment, when the colony on all the different concentrations is approaching the edge of the Petri dish. The successive curves are more or less equidistant from each other—in other words, all the colonies are growing at a uniform linear rate throughout the duration of the experiment.

In the case of the strain A, however (Fig. 1), matters are somewhat different. What has been said above as regards *Fusarium acuminatum* applies to strain A only for the cultures on P.E./4 and lower concentrations.

There is from about the sixth day a slight falling off in linear growth from P.E./4 towards the right, but this difference has not always been observed, and it is always small. The drop in linear growth on passing from the
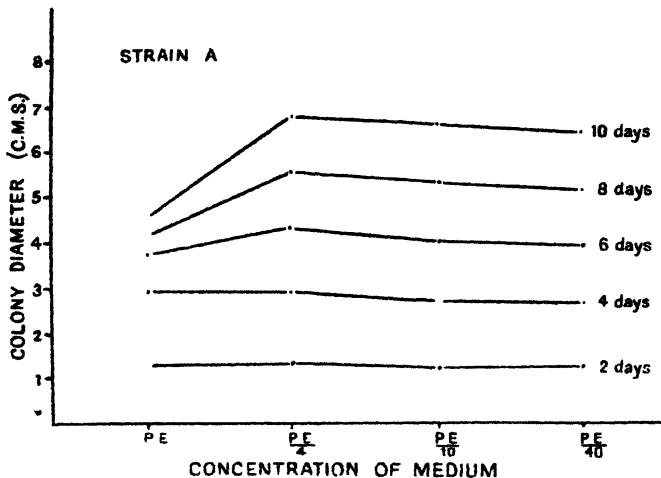
FIG. 1. Growth of *Fusarium*, strain A, on various concentrations of potato agar.
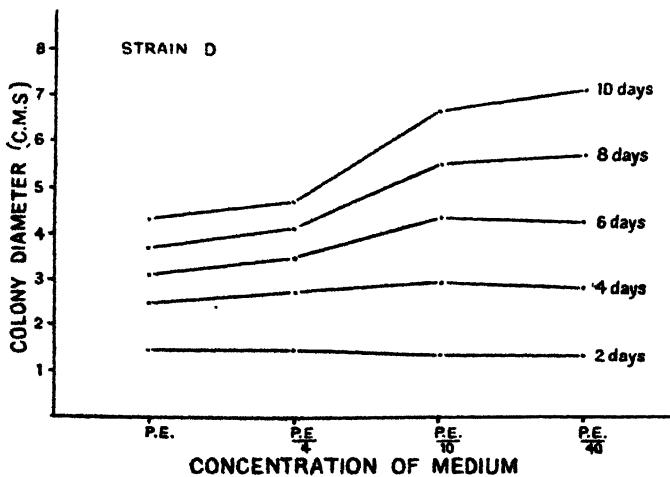
FIG. 2. Growth of *Fusarium*, strain D, on various concentrations of potato agar.

concentration P.E./4 to P.E. is, however, very marked. This effect is not due to a slower intrinsic rate of growth, but to a falling off in the rate from about the fourth day. The result of this is that whereas the colonies on P.E./4 and lower concentrations go on growing at sensibly uniform rate until they approach the edge of the dish, the colonies on P.E. show a pro-

gressively slower and slower rate of expansion, and finally reach a standstill, it may be at a considerable distance from the edge of the plate.

One may express these results as follows: *Fusarium acuminatum* on the various concentrations employed shows unstaled marginal growth. Strain A shows distinct marginal staling on the strongest medium, but somewhere between the concentrations represented by P.E. and P.E./4 this staled type of growth gives place to the unstaled type, and the latter is shown at all the further dilutions. In other words, to get the unstaled type of growth with strain A, one must dilute the particular potato decoction to some point between P.E. and P.E./4.

. Fig. 2, representing strain D, illustrates the case where the staled type
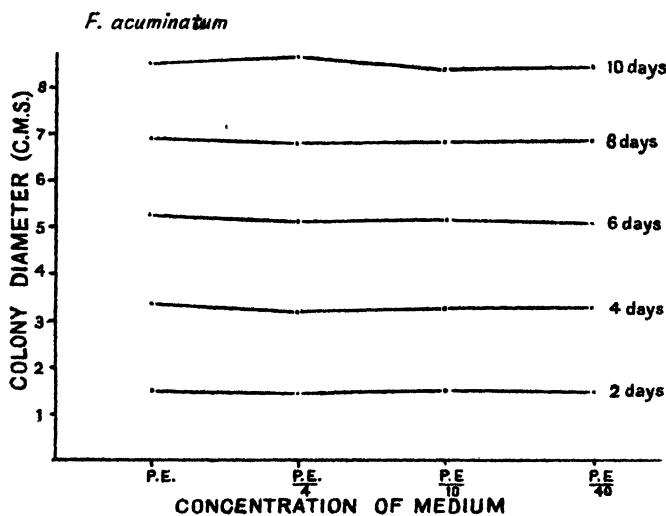
FIG. 3.   Growth of *Fusarium acuminatum* on various concentrations of potato agar.

of growth is still obtained at a dilution of P.E./4, and to a less extent at a dilution P.E./10.

It appears, then, that the amount of the staling effect shown by these fungi is a function of the concentration of the medium. If on a certain concentration of potato agar a particular strain shows staling at the margin of the colony, this effect can be removed by diluting the medium sufficiently. The difference from the point of view of growth between strains A and D is that in the case of the latter a greater degree of dilution is necessary for this purpose. Viewed in this light, strain D would be described as a more strongly staling form than A, while *Fusarium acuminatum* shows this effect least of all. One would expect that if the last-mentioned fungus were grown on a medium more concentrated than that represented by P.E. it would also show the staled type of growth. This was proved to be the case, viz. on a medium containing 400 grm. potato to the litre.

By the early experiments along these lines, it was shown that a representative set of strains could be arranged in series of graded staling capacity. Such a series was the following, beginning at the one showing least staling : *Fusarium acuminatum* ; strain A ; strain F ; strains B, E, and C_3 ; strain D.

In successive experiments, with potato agar as medium, it was found that though the different strains always arranged themselves in the same order, the point at which the staled type of growth gave way to the unstaled type on dilution varied somewhat with successive batches of the medium. Thus, on some batches of potato agar strain A staled much more distinctly than on others, suggesting that successive lots of medium were not uniform in character.[1] It was decided therefore to attempt to prepare a synthetic medium which would give approximately the same type of growth as that obtained on potato agar, with a view of adopting it for the study of the relationships of these closely allied forms.

Before proceeding to describe the manner in which this synthetic medium was arrived at, it will be convenient here to outline certain generalizations of the results already put forward, and to mention some particulars by way of clearing the ground.

A feature of the growth-curves shown in Figs. 1–3 is that each strain shows the same rate of growth in the early stages on all the dilutions of the medium tested.   During the course of this work many thousand measurements have been made, dealing with practically all the *Fusarium* strains under consideration here and using a large number of media, chiefly synthetic, of widely varied composition.   As a result it can be stated that, provided the medium is not unduly concentrated and possesses no deleterious properties. such as, for example, too high acidity, the initial rate of radial growth[2] of a particular *Fusarium* strain is the same, or very approximately the same, for all modifications and dilutions of these media.   It is true that spore germination is somewhat retarded in the more concentrated media, but this effect is not noticeable in the macroscopic measurement of the colonies produced.   Two illustrations will show to what an extent the initial rate of growth is independent of the composition of the medium. The synthetic medium which is a fair representation of potato agar for the growth of these *Fusaria* has been varied from a concentration which may be represented as 4 down to one of 1/40, the relative concentration of each

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[1] Potato agar is prepared in this laboratory by boiling 200 grm. potato in water, squeezing through muslin, and making up to a litre.   In practice it is troublesome to press the whole of the potato tissue through the muslin, so that a certain amount of coarse residue is discarded.   It is obvious that this method of preparation will lead to variations in different samples.   Over and above this there are the seasonal and varietal variations of the potatoes themselves.

[2] The best way to determine the initial rate of growth is to take the reading from the second to the third day (i. e. assuming that the experiment is carried out at ordinary temperatures).   By this means one eliminates irregularities due to different size of inocula, lag in germination due to somewhat high concentration of medium or to attenuation of spores, &c.

constituent has been varied within wide limits, but none of these modifications of the medium sensibly alters the initial growth-rate of the fungus. Another example is the following: Richards's solution agar would be described as a rich concentrated nutrient, nevertheless the initial rate of growth of a given *Fusarium* strain on this medium is practically the same as that on plain agar merely. The effect of the nutrient is shown, *not on the rate of spread but on the density of the colony*. Temperature, it may be added, is the one environmental factor which strongly affects the growth-rate.

A further generalization is possible. All the *Fusarium* strains, numbering about forty, which are the subject of this investigation, have the same initial growth-rate. Some of these strains would be described as having limited growth, others as having unlimited growth, i. e. some form colonies which stop growing comparatively early, others grow at undiminished rate to the edge of the plate. Nevertheless, though the resultant colony size is so different in the different strains, there is no appreciable difference between the growth-rates in the early stages. This initial growth-rate, which is maintained until staling effects come into play, may be looked upon as the *intrinsic rate of growth* of the fungus, being as it is independent within very wide limits of the composition and concentration of the medium. The fact that all the strains in cultivation have the same intrinsic rate of growth is interesting, in view of the evidence which will be presented later of their genetic connexion with each other.

The application of the conception of an intrinsic rate of growth to some other fungi would not be as easy as in the case of *Fusarium*. Fungal growth is in general characterized by an initial lag, even after allowance is made for the period required for germination proper. With these *Fusarium* strains this lag effect is comparatively small, so that the fungus reaches its limiting growth-rate in a day or so. The case of *Botrytis cinerea* is somewhat different. Here the fungus under certain conditions will continue to show accelerated growth over several days, and may reach the limits of an ordinary (5 in.) Petri dish before the limiting rate has been reached. With such a fungus the conception of an intrinsic growth-rate is of less applicability.

It was shown above that the effect of increasing the concentration of the nutrient was to diminish the size (diameter) of the colony as measured after, say, about ten days. It was found that on further concentration of the medium a stage was reached at which the diameter again increased. Also the concentration at which this rise in the curve took place was lower for a strain such as D than for A. This is represented diagrammatically in Fig. 4. Thus it is not unnatural to suggest that the essential feature of these growth-curves is that they are of the same general shape, but that they differ simply as regards their position along the *x*-axis. If one starts with

a strong medium like Richards's solution and makes successive dilutions, the complete curve shown in Fig. 4 for strain D is readily obtained.

On theoretical grounds one could anticipate that if the curve were traced still farther to the left it would again descend, for there must be
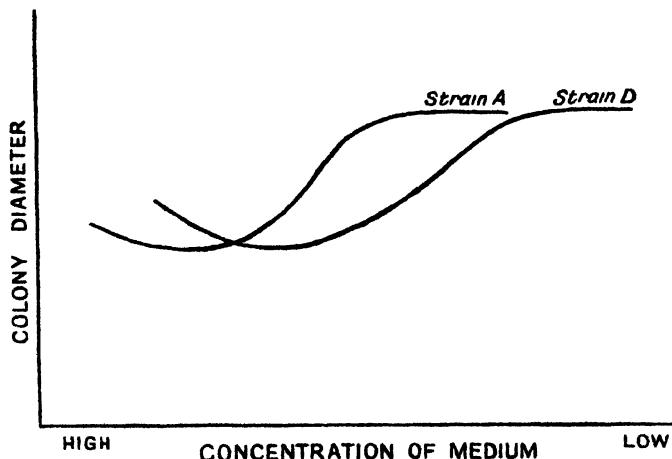
FIG. 4. Diagrammatic representation of the growth of a weakly staling (A) and a strongly staling (D) strain of *Fusarium* on various concentrations of a nutrient medium.
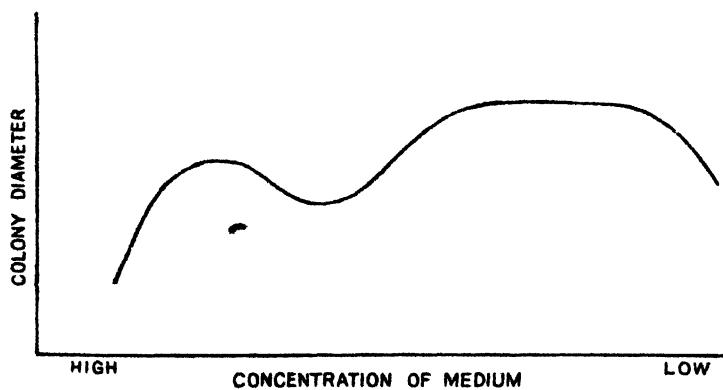
FIG. 5. Generalized curve of colony growth over a wide range of concentration of medium.

a concentration at which osmotic difficulties come into play. Such a concentration is beyond the region of ordinary nutrient media, and no attempt has been made to extend the curve in this direction. Again, the weakest medium that has been used has been plain agar. As commercial agar is not pure, such a medium has a definite though small nutritive value, and it is probable that if a geling medium of no nutritive value at all were

obtained the curves in Fig. 4 would also descend on the right. Thus the complete curve representing colony size against concentration of the medium would have such a form as is represented in Fig. 5. The main feature of interest about this curve is the presence of the distinct minimum point in the middle. For the case of these *Fusarium* strains the most important part of this general curve is from about the minimum point towards the right, as it is in this region in general that these strains show their maximum sporulation.

It is interesting to note how such a fungus as *Botrytis cinerea* behaves in this connexion. With potato agar of normal strength and its dilutions, the *Fusarium* strains give that portion of the curve extending to the right from about the minimum point. On the same series of media, the colony size with *Botrytis cinerea* diminishes continuously with dilution. In particular, *Botrytis*, which shows no staling at all on potato agar, stales strongly on plain agar, this being an exactly opposite behaviour to that of some of the *Fusarium* strains. It is suggested that the curve of colony growth for *Botrytis* would be got by displacing the *Fusarium* curve still farther to the left. However, no attempt has been made to test this point.

Certain details relative to the carrying out of these growth measurements may be referred to here. In setting up the colonies for measurement, as far as possible equal masses of spores were used. Even when the inocula differed very considerably in amount of spores added there was very little, if any, effect on the subsequent growth. The size of the inoculum within reasonable limits is a matter of no consequence.

It is inadvisable to continue readings of diameter when the colony approaches closely (say less than $\frac{1}{2}$ in.) to the edge of the Petri dish. The nearness of the glass, by preventing the dilution of staling products which otherwise would have diffused away, has the effect of slowing down growth, in circumstances where no slowing down would be shown in a wider dish.

The depth of the medium is a matter of some consequence. For a considerable time deep-poured (about 1 cm.) and shallow-poured (about $\frac{1}{4}$ cm.) plates were systematically used in all experiments. The curves obtained in the two cases are essentially the same as is shown in Fig. 6, which illustrates the main features. The continuous curve represents the colony diameter of strain A grown on a series of potato media (P.M. = potato mush, a thick medium containing 400 grm. potato to the litre). The media are arranged in descending order of concentration from the left and are placed arbitrarily at equal distances along the x-axis (though the medium P.M./2 is only very slightly stronger than P.E.). As has been shown already, the non-staling type of growth is given towards the right of the curve and the staling type towards the left. The dotted curve represents the growth on shallow plates. In the unstaled region the curves are practically coincident: in the staled region the shallow plates show more pronounced staling than the

deep ones. Thus the curves are of the same shape, but the dip is more pronounced in the shallow plates.

In the region of concentration at which the unstaled type of growth passes over into the staled, an interesting crossing of the curves has
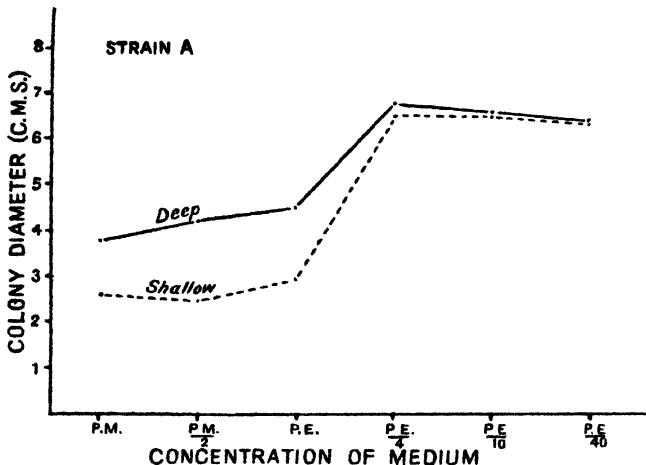
FIG. 6. Effect of depth of medium on amount of growth (superficial) at various concentrations of nutrient medium.
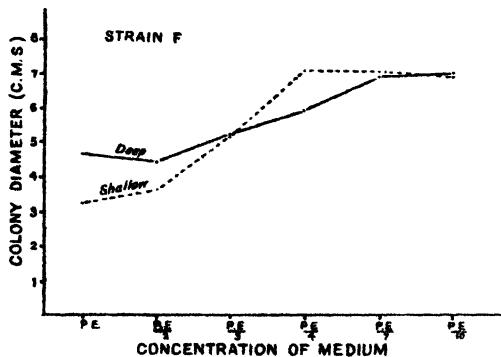
FIG. 7. Crossing of growth-curves for deep and shallow media at the transition region between staling and non-staling types of growth.

frequently been observed, especially in the case of certain strains. Fig. 7 illustrates such an effect. Between the concentrations P.E./7 and P.E./4, the deep plates begin to show the staling effect, whereas with the shallow plates a somewhat higher concentration has to be reached to effect this. Thus within narrow limits shallowness of the medium is equivalent to dilution. In line with this result one finds that in the region of concentration, where the staled type of growth is passing over to the unstaled, very uneven results are obtained if one deals with plates in which the medium is shallow. One plate may show a strongly staled colony, a control to this

may show a widely spread unstaled colony, while cases have frequently been met with in which one side of the colony was staled and the other unstaled, thus giving rise to a markedly eccentric colony. In such cases it was seen on testing that the more spreading side was the shallower one.

These considerations constitute an objection to the use of shallow layers of medium in growth work. Further considerations pointing in the same direction are that in deep-poured plates the percentage variation in depth of plating (which is done simply by judgement) is less than in shallow-poured ones, and again that variations in depth due to irregularities in the bottoms of the Petri dishes are of less significance in plates which are poured deep. Hence it is recommended that in all work entailing the measurement of colony diameter the layers of medium in the plates should be reasonably deep ; a depth of about ¾ to 1 cm. is suggested.

C. Preparation of a Synthetic Medium approximately equivalent to Potato Agar.

As stated above, potato agar, though very suitable for the culture of the *Fusarium* strains by reason of its capacity in general to induce sporulation, is liable to vary in composition, so that a synthetic substitute, if it could be found, would prove of great advantage. In proceeding to compound such a medium, a start was made from a typical potato analysis. The average composition of a number of potato tubers as given in Thorpe's ' Dictionary of Applied Chemistry ' (art. ' Potato ') is as follows :

Water, 75·8 %

	Fat	0·2 %	
	N-containing	1·8 %	{ 0·9 % globulin and proteose. { 0·9 % amino acid.
Solids, 24·2 %	N-free	20·5 %	{ 19·5 % starch. { 1 % sugar.
	Fibre	0·7 %	
	Ash	1 %	

As regards the last constituent, 100 grm. ash contain 60·3 grm. K_2O, 2·6 Na_2O, 2·6 CaO, 4·7 MgO, 1·2 Fe_2O_3, 17·3 P_2O_5, 6·4 SO_3, 2·0 SiO_2, 3·0 Cl_2.

Assuming this composition, a decoction of 200 grm. potato to the litre contains in the litre the following :

3·6 grm. nitrogenous substance, half as protein or proteose, half as amino acid.

0·4 grm. fat.

41 grm. carbohydrate (39 grm. starch, 2 grm. sugar).

1·4 grm. fibre.

2 grm. ash.

In preparing a trial solution, the fat and fibre constituents of the organic part were ignored, as were also the calcium, sodium, and silicon constituents

of the ash. The latter were omitted as there is a large body of evidence that they are in general non-essential elements for fungal growth.

The remaining constituents of the potato were then added in the following forms and proportions, the latter being calculated to give as closely as possible the quantities required by the analysis:

> 1·8 grm. peptone,
> 1·8 grm. asparagin,
> 2 grm. glucose,
> 40 grm. potato starch,
> 0·15 grm. KCl,
> 0·75 grm. $MgSO_4$, 7 H_2O,
> 1·35 grm. K_3PO_4,
> Trace $FeCl_3$,
> 15 grm. agar powder ; all made up to 1 litre.

The peptone and asparagin were assumed to be equivalent to the more complex and the simpler parts respectively of the N-containing constituent of the potato. The potassium phosphate was added as the neutral (in reality faintly alkaline) salt as the natural potato decoction is approximately neutral.

To begin with, the incorporation of 40 grm. potato starch to the litre was somewhat troublesome, so that this quantity was reduced from the start and 10 grm. tried instead.

All the original strains, six in number, and some other species of *Fusarium* were grown on plates of this medium side by side with cultures of the same on potato agar of the usual strength. The resemblance was strikingly close—both media gave in a general way the same type of mycelial growth in the various strains, the same kind of staling effects, and similar amounts of sporulation. Thus the replacement of the potato agar by a synthetic medium giving similar fungal reactions appeared to be quite simply effected.

It seemed probable, however, that this synthetic medium was unnecessarily complicated, so that attempts were made to see how far it could be simplified without losing its character of being a fair substitute for the natural decoction. Experiments were therefore carried out in which the growth of two strains, A and C_3, on the full medium was compared with that on media in which each constituent in turn was omitted. The growth-rates of the colonies were determined at intervals and the general appearance noted in detail. The results were very clearly defined. The colonies on the full medium were not distinguishable in any way from those in which the following constituents were omitted: KCl, $FeCl_3$, $MgSO_4$.

As the essential element potassium is provided for by the potassium phosphate, and as chlorine is not an essential element, the KCl constituent was dropped without further consideration.

Though iron is considered to be an essential element, yet it is only necessary in minimal concentration. In the present case it was obvious that the needs of the fungus for iron were satisfied by the impurities present in the chemicals. Even in flask cultures on a liquid substratum where the constituent most likely to contain impurities, viz. the agar, is absent it is very difficult with ordinary chemicals to demonstrate that the omission to add a trace of iron makes any difference to the growth, either quantitatively or qualitatively. For this reason the $FeCl_3$ constituent was discarded from the synthetic solution.

The case of the magnesium sulphate was somewhat different. Both the magnesium and the sulphur are reckoned to be essential for growth. Nevertheless, no difference in growth on the synthetic medium with agar could be demonstrated when the magnesium sulphate was omitted. At the same time experiments with the synthetic medium without agar in flasks showed that the omission of magnesium sulphate resulted in almost complete suppression of growth. The apparent discrepancy was explained when it was found that the agar powder contains appreciable quantities of magnesium, as was shown by the formation of a definite precipitate when ammoniacal phosphate was added either to an agar gel or to the washings of agar powder. In view of this evidence of the necessity of magnesium sulphate in appreciable quantity, and therefore of the inadvisability of supplying it as an incidental impurity, this constituent of the synthetic medium was retained.

The omission of potassium phosphate produced a marked effect on the appearance of the fungus, namely, very feeble growth with pronounced staling and the development of a yellow colour which was absent in the colonies on the full medium. It was obvious that this constituent was essential to the synthetic medium.

As regards the four remaining constituents, glucose, starch, asparagin, and peptone, the omission of peptone and of starch produced the least effect on the growth-form of the colony. The plates lacking in peptone showed much the same appearance as those on the full medium, being somewhat less staled and showing somewhat heavier sporulation over the central part of the colony. This was the case of strain A. With strain C_3 there was no obvious difference between the growths on the full medium and on the one lacking in peptone. In view of these small effects and of the variable nature of peptone preparations, this constituent was discarded from the standard medium.

The effect of leaving out the starch constituent also was not great. It was shown chiefly in a diminution in the amount of sporulation. As regards the effect on colony size, a small effect was noted in the case of strain A, none in the case of strain C_3. This constituent was therefore also dropped, though it will be shown that its use was continued for certain purposes.

The omission of the glucose constituent produced a considerable change —viz. almost total suppression of aerial mycelium. Other characters were not markedly changed. This constituent was retained.

The most marked change of all was brought about by leaving out the asparagin, the nitrogen source in this case being simply peptone. The appearance of the colonies was entirely different, marked features being the strong development of colour in the medium and the abnormal appearance of the spores. These plates presented in fact the typical appearance, as it was afterwards shown, of growth on a medium in which the amount of nitrogen bears a low ratio to the amount of the carbon constituent. Obviously asparagin was an important constituent.

That each of the four constituents last dealt with does influence to some extent the course of metabolism in the colony was shown by following the development of alkalinity in the colonies. It then appeared that the media arranged themselves in the following order, beginning at the one showing strongest alkalinity: Medium minus starch—medium minus glucose—full medium—medium minus peptone—medium minus asparagin; the last one remaining neutral or approximately so. It is obvious from this series that the nitrogenous constituents are responsible for the development of alkalinity, while the glucose and starch tend to counteract this.

On the basis of these results, the following was adopted as a standard medium,[1] the quantities of some of the constituents being slightly modified as compared with those given on p. 385:

Glucose	2	grm.
Asparagin	2	,,
K_3PO_4	1·25	,,
$MgSO_4, 7H_2O$	0·75	,,
Agar	15	,,
Water	1 litre	

This medium was made the basis for studying the effect of the composition of the nutrient medium on the growth-form of the various strains. Starting from the above formula it was varied both as regards concentration, the ratio between the constituents being kept constant, and as regards composition, the ratio of the three most important constituents being varied in different directions. This experimental work has constituted the main part of the present research, and a large body of data bearing on the response of

[1] This medium, though somewhat too dilute and with too low a glucose/asparagin ratio, is a very fair substitute for ordinary potato agar (200 grm. to the litre) for the culture of *Fusarium*. It gives good results with many other fungi, but with some it is not even approximately equivalent to potato agar. *Botrytis cinerea, Thielavia basicola,* the fungus of internal boll disease of cotton, and some others have been found to grow feebly on this medium as compared with the natural decoction. Possibly in these cases the simplification has been carried too far. Further work on this subject is in progress.

growth-form to cultural conditions has been accumulated. The macroscopic characters will be dealt with in the present paper: the modifications of the microscopic features, which have been studied specially in relation to the spores, will be described in a later one.

D. Effect of Concentration of Medium upon Growth-form.

In describing the effect of mere change of concentration of a medium, it is proposed first of all to describe the course of development of a colony of a particular strain on the standard medium as given above, and then to consider how the developmental features are affected when the medium is used in a more dilute or more concentrated form.

Strain A. This strain on the standard medium and at ordinary temperatures (17° C.) grows approximately at a uniformly linear rate until it covers the whole plate. In the young stages the central part of the colony is covered with a scanty aerial mycelium, the marginal zone being pellucid. During the whole course of growth this pellucid marginal zone persists. The fate of the aerial mycelium in the central region depends ·on certain environmental factors, particularly on light.

When the cultures are kept in alternating day and night, the aerial mycelium is short-lived, so that one finds, as the colony grows, that the scanty aerial mycelium in the centre disappears and in its place are found pustules of spores (sporodochia). The latter show in this strain, though not in strains otherwise very similar to it, a distinct tendency to zonal arrangement, a phenomenon in this case definitely correlated with the alternation of light and darkness, i. e. a new zone of spores is formed corresponding to each succession of day and night. When similar cultures are grown in the dark, the aerial mycelium formed is much more lasting, so that the colony at the time when it has covered the plate shows aerial mycelium over its whole surface except in a zone behind the growing edge. The persistence of the aerial mycelium in this case is reflected in a corresponding reduction in the amount of sporulation ; indeed, by carefully excluding light at all times during growth, almost sterile colonies of this race can be obtained, though some of the mycelium so formed is still capable of developing spores on subsequent exposure to light.

An instructive experiment in this connexion is to grow the colonies in darkness and to expose them, say at three-day intervals, to light for a few minutes. These colonies will then produce rings of spores at intervals corresponding to three days' growth, showing that a short exposure to light is all that is necessary for the stimulation of sporing and that the actual formation of the spores will then take place in the dark. The radii of the spore rings are a little less than were the radii of the colonies at the time of exposure to light, whence it follows that the region of greatest sensitivity is a short distance behind the growing margin of the colony.

The rate of growth of the colony is not appreciably affected by variation of the conditions of illumination.

The general sequence of growth appearances in a culture exposed to the light is thus as follows : As the colony grows it shows a pellucid margin, followed by a zone of scanty aerial mycelium. The latter zone grows in front and disappears behind at more or less the same rate to give place to a sporodochial region free from aerial mycelium. When fully developed, such a colony shows an area of more or less continuous sporulation in the centre, beyond which is a succession of spore zones, each one corresponding to one day's growth. These zones continue outwards for a distance which depends to some extent on the depth of the medium. The intensity of sporulation tends to diminish from the centre outwards.

When successive dilutions of the standard medium are tested, the following effects are observed : There is no appreciable change in the rate of spread throughout the dilution series. The amount of transient aerial mycelium which is formed becomes progressively less and less down the series, and at a certain stage of dilution the colony is pellucid throughout its whole period of growth. *Pari passu* with this reduction in the aerial mycelium, there is a reduction in the intensity and in the spread of the spore-bearing region. This is shown by the reduction of the number of visible spore rings and by the contraction of the central region of more or less continuous sporulation. On further dilution, visible sporulation is restricted to a small group of pustules round the point of inoculation. When the state of extreme dilution represented by plain agar is reached, there is no visible sporulation at all, though small scattered groups of spores can still be demonstrated under the microscope.

On concentrating the standard medium, the converse series of changes is produced. The intensity of aerial mycelium formation is increased, and when a concentration is reached which produces staling of the growing margin, the zone of aerial mycelium extends up to the margin itself. Within the aerial mycelium zone is a spore-bearing central region which has followed the disappearance of the aerial mycelium which once existed there. The zone of aerial mycelium framing the stale culture is of a quasi-permanent nature and only slowly disappears : such a colony shows little or no further spread. The region of sporulation is therefore also contracted as compared with the corresponding region of the colony grown on the standard medium. Nevertheless, correlated with the greater intensity of aerial mycelium, the density of sporulation increases with the concentration of the nutrient, so that the central region of the colony, instead of showing a succession of spore zones, consists of a more or less continuous layer of spores. With further increase in concentration of the nutrient the proportion of aerial mycelium which goes down becomes less and less, so that the spore-bearing region in the centre becomes contracted and finally disappears.

The colony now shows the following features : Mycelium in the form of a strong felt ; the whole colony covered by persistent aerial mycelium ; spores absent or at most appearing late in small irregularly scattered masses on top of the aerial mycelium.

The above description may be summarized as follows :

The effect of concentrating the nutrient from extreme dilution (i. e. plain agar) to the concentration represented by the standard medium is to increase the density and spread of the sporulation. All the colonies are unstaled and there is no permanent aerial mycelium. With further concentration of the medium, growth at the margin becomes limited, a zone of permanent aerial mycelium remains at the margin, and this mycelial zone with further concentration spreads inwards and obliterates the spore-bearing region in the centre of the colony.[1] The last stage of the process of concentration is a sterile colony with dense weft of mycelium.

The above summary applies to all the strains under consideration here. The differences as between strain and strain are in respect of the intensity of the various appearances and of the particular concentration of the medium at which they are shown. These points will be illustrated by a brief consideration of three other strains.

Strain C 3. This strain is characterized, as compared with strain A, by (1) more intense sporulation, (2) more intense staling. Its capacity for stronger sporulation is reflected in (1) its production of visible spore masses even on plain agar, (2) its showing denser sporing features at the various concentrations, (3) its continuing to sporulate freely at concentrations beyond that which gives only sterile mycelium in the case of strain A. Its stronger staling capacity is shown by the fact that it forms colonies of limited size when a concentration of about half that of the standard medium is reached. Whereas strain A at this concentration shows a series of rings extending over almost the whole plate, strain C 3 takes the form of a small colony which is covered by a continuous mass of spores, with a slight fringe of aerial mycelium round the margin. Apart from these differences in degree, the succession of appearances shown on the various concentrations of medium is exactly the same as that described for strain A.

Strain D. This is characterized by its strongly staling capacity and by somewhat feeble sporulation. On the standard medium it forms colonies of limited size with sporodochia confined to the neighbourhood of the centre. Beyond this is a zone with neither spores nor aerial mycelium, and then comes a dense zone of more or less persistent aerial mycelium round the

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[1] Over a fairly wide range of media, this persistent aerial mycelium can be stimulated to form spores by mechanical disturbance. For this purpose all that is necessary is to stroke the mycelium with a wire. Along the line of the stroke spores will in many cases form freely under circumstances where they would have formed sparingly, if at all, in the absence of mechanical disturbance. In order to be effective the stroking must be done before the mycelium has become too old.

outer region of the colony. This aerial mycelium goes down in course of time and in its place is left a single strong continuous zone of spores. On dilution of the medium the formation of aerial mycelium gets less and less ; the continuous marginal zone of spores comes to be represented by a zone of separate sporodochia, and on further dilution this spore zone disappears altogether and the strain now has the appearance of a feebly sporing edition of strain A at the same concentration of medium.

On concentrating the standard medium one passes very soon to a growth-form which has permanent aerial mycelium throughout, apart from a pustule of spores at the centre and a more or less interrupted thin ring of spores lying on the marginal aerial mycelium. With a little further concentration of the medium all sporulation disappears. The colonies of this and similar strains on the more concentrated media form a compact more or less wrinkled mat of mycelium and show the maximum tendency, within the group of Fusariums under consideration, to the formation of sclerotia. Contrasted in this respect with these mycelial strains are the pionnotal strains of which D_3 is a representative. These have not been seen to show any indication of sclerotium formation on any medium.

Strain D 3. This is similar to strain C 3 in staling capacity, but sporulates still more strongly. On the standard medium there is never more than a trace of aerial mycelium at any stage, and in the fully-grown colony nothing is seen by the naked eye but a continuous layer of spores (a so-called *pionnotes*). Dilution of the medium simply has the effect of thinning out this layer of spores and, when a sufficient degree of dilution is reached, of producing a non-staling type of colony. At the extreme dilution represented by plain agar, this fungus still shows vigorous sporulation, the colonies displaying obviously the pinkish-orange colour indicative of the presence of spores.

On passing upwards from the standard medium there is no change in the character of the colonies apart from increasing intensity of the spore layer. Even at a concentration four times that of the standard medium, the pionnotal form still persists over the whole surface of the colony. It is only on such comparatively highly concentrated media as Richards's solution, or potato agar to which 5 to 10 per cent. cane sugar is added, that one obtains even a trace of persistent aerial mycelium. This strain and others of the same type have never been obtained at all in the wholly mycelial form.

E. General Discussion on the Effect of the Concentration of the Medium upon Growth-Form.

The above descriptions referring to particular type strains apply in a general way to all the strains so far examined with regard to the effect of concentration of medium upon growth-form. It is clear that the same

principle applies to all.   Starting from the extreme dilution represented by plain agar, one finds that increase of concentration is accompanied up to a certain point by increase in sporulation.   This increase of sporulation is shown both by greater intensity at any point and by greater spread over the surface of the colony.   But by and by a stage is reached where increasing concentration of nutrient is accompanied by increasing stability of the aerial mycelium.   At such a concentration the spread of the sporulating region is reduced, and at a sufficiently high concentration the spore-bearing region is abolished altogether.   Thus, with very dilute media, increase in concentration of medium means increase in amount of mycelium and in amount of sporulation ; when a certain concentration is reached, further increase, while leading to increase of mycelium, results in diminution of sporulation. The point at which the positive correlation between concentration of medium changes to a negative one is where persistent aerial mycelium forms as a thin zone round the edge of the colony.   For most of the forms dealt with here this is somewhere near the point at which the colonies show the transition from the non-staling to the staling type of growth.   Thus it is in the neighbourhood of this point that these strains in general show their maximum sporulation.   This rule applies especially to the poorly sporing mycelial types and to the moderately sporing sporodochial type of which strain A is an example. With the intensely sporing pionnotal forms the rule does not apply, as they continue to show increased sporulation long after the concentration at which marginal staling takes place has been passed.

The observations described above point to a strong connexion between sporulation and the disappearance of the ephemeral aerial mycelium which preceded the spores.   This disappearance is obviously a case of autolysis. Though the autolysis of the aerial mycelium is the salient feature observed, one may safely postulate the same autolytic effects in the general mycelium of the fungus, immersed or otherwise.   Definite evidence in favour of this can be adduced.   Thus if one estimates roughly the amount of mycelial weft by taking its mechanical resistance, one finds that the mycelial weft below a strongly sporing strain is negligible as compared with a feebly sporing strain.   Even in the same culture, when there is a strongly sporing central area with a marginal mycelial fringe, there is a much less resistant mycelial layer below the area of spores than below the area of aerial mycelium. There is thus a strong suggestion that autolysis of the mycelium and sporulation are correlated phenomena.

In studying the conditions producing autolysis of the mycelium one would probably require to consider as factors both the exhaustion of the nutrient medium and the presence of staling substances.   That the presence of the latter is not the direct cause of autolysis is shown by the fact that, on comparing strain with strain, there is no correlation between staling and

sporulating capacities. Thus there are strongly staling forms which sporulate intensely and others which do so feebly, and similarly among the feebly staling strains there is a wide range of sporulating capacity. It seems probable that exhaustion of the nutrient is the factor which initiates autolysis (and therefore sporulation), but that staling substances play a part inasmuch as they provide a suitable reaction in the medium for the autolysis to proceed. The optimum concentration for sporulation would, according to this view, be the strongest which allowed of exhaustion of the nutrient without at the same time producing a concentration of staling substances inhibitory to the autolytic process. Below this concentration sporulation would be less on account of the reduced amount of energy available; at higher concentrations it would also diminish, as an inhibitory concentration of staling products would appear in the culture before exhaustion of the medium had proceeded far enough. For the elucidation of this problem, however, a quantitative study of the rate of assimilation in fungal cultures is obviously required.

F. Effect of Composition of Medium upon Growth-form.

The standard medium has been varied in respect of the three important constituents, phosphate, asparagin, and carbohydrate. These will now be dealt with in the order stated.

I. *The Phosphatic Constituent.*

The phosphate ingredient contains also potassium, and it is undoubted that this also is of importance. On comparing the type of growth obtained on media which differed as follows:

> A, with potassium phosphate,
> B, with equivalent sodium phosphate,
> C, without phosphate,

it was found that the difference in growth-form between A and B was slight as compared with that between either A and C or B and C. The only visible difference between A and B consisted in somewhat reduced sporulation in the latter. This is in agreement with the general view that potassium plays some part in the reproductive process. As the chemicals used were not of a fine grade of purity no hard and fast conclusion can be drawn from this result other than that the effects produced on varying the potassium phosphate constituent are referable chiefly to the phosphate radical. The result already quoted (p. 385), that the addition of a certain amount of potassium chloride to the synthetic medium makes no apparent difference to the growth, indicates clearly that the growth-form of the fungus is fairly insensitive to variation in the potassium content in the medium.

The following experiment illustrates the effect of reducing the phos-phatic constituent in the standard medium. The phosphate (K_3PO_4) was in the proportions given below, the other constituents being as in the standard medium : I, 0·1 per cent. ; II, 0·05 per cent.; III, 0·02 per cent. ; IV, 0·01 per cent. ; V, none.

Strain A was grown on these media at laboratory temperature (*c.* 15° C.). On I and II there was no staling ; there was only evanescent marginal mycelium ; sporulation was in rings and over most of each plate. On III the amount of sporulation was definitely reduced ; staling began to appear after about fourteen days ; the marginal fringe of aerial mycelium definitely persisted longer than in I and II. On IV strong staling appeared from about the tenth day ; sporulation was restricted to the neighbourhood of the centre of the colony ; the marginal fringe of aerial mycelium was well developed and persistent. On V a thin colony was formed, showing staling effects even on the seventh day and devoid of aerial mycelium and spores.

The effects of reducing the phosphate content of the medium are thus : (1) the intensification of staling effects ; (2) the reduction of sporulation, both in respect of spread and intensity ; (3) the increased stability of aerial mycelium down to a certain degree of dilution, after which further reduction in the amount of phosphate leads finally to a meagre colony devoid of aerial mycelium.

The colonies on medium V have a general yellow colour. The mean-ing of this effect will be dealt with later. It may be remarked that the growth-form of strain A (sporodochial) shown on the medium IV (phos-phate 0·01 per cent.) is practically the same as that shown by strain D (mycelial) on medium I (phosphate 0·1 per cent.).

Effect of increasing the concentration of neutral phosphate. The behaviour of two strains, A and D, will serve to illustrate this effect.

Strain A. When grown in light on the standard medium (phosphate 0·125 per cent.) this strain shows unstaled colonies with practically no aerial mycelium and with distinct zonal arrangement of the spores. Increase in the amount of neutral phosphate has, up to a certain point, no effect on the rate of spread of the colonies, these being all unstaled. The only effect seen is in an increase in the intensity of sporulation. The following experi-mental data illustrate this point: The colonies were incubated at 20° C. Measurements of diameter of the colonies were made on the third day and then on every second day. The resultant colonies showed rings of spores corresponding to the number of exposures to light. The following diagram (Fig. 8) represents to scale the distribution of sporing in the colonies in radial section, the heavy lines representing the spore zones and the numbers being the distances from the centre.

The average width of the spore zones in the first case was about

0·2 cm.; with medium amount of phosphate, 0·4 cm.; and with the highest phosphate, 0·5 cm. The colonies with the highest phosphate were almost a continuous mass of spores.

When in the standard medium the concentration of phosphate reaches about 2 per cent., the rate of spread of the colony begins to diminish. This is not due to any staling; the growth is unstaled throughout, but the intrinsic rate of growth is reduced by the increased concentration of phosphate.

Strain D. This strain, when grown on the standard medium, is of the mycelial type and shows staling. Increase of phosphate causes decrease of aerial mycelium, increase of sporulation, and increased spread of the colony. Thus by increasing the phosphate constituent the poorly sporing mycelial
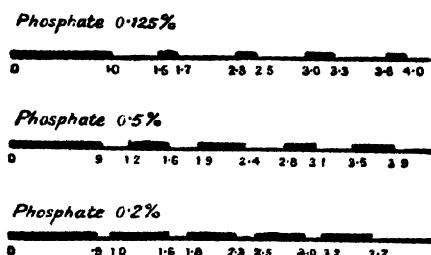
FIG. 8. Diagrammatic, illustrating effect of increased concentration of neutral phosphate in intensifying sporulation. For further explanation, see text.

strain D can be made to take on approximately the characters shown by the sporodochial strain A on the standard medium.

The effect of phosphate content of the medium has been tested on a number of other strains and the general conclusions appear to be applicable to all. On a medium with minimal phosphate the colonies formed are staled, and devoid both of aerial mycelium and spores. With increase in the amount of neutral phosphate, aerial mycelium appears until a certain optimum is reached, which is lower in the case of a freely sporing strain than with one of the mycelial type. With further increase of phosphate the colonies pass over to the non-staling type devoid of aerial mycelium, and this growth-form continues to be shown up to the highest concentration tested (2 per cent.). In the neighbourhood of the latter concentration the intrinsic rate of growth of the fungus begins to diminish. Density of sporulation increases continuously with increase of phosphate up to the limits tested.

The differences as between strain and strain consist in the concentration of phosphate at which the various transitions from one growth-form to another take place. The same order of succession is shown by all.

Comparison of neutral and acid phosphates.   If in the standard medium the neutral phosphate is replaced by an equivalent weight of either of the acid potassium phosphates, the effect on the growth-form is small. This is easily understood.   The medium is such that the colony develops an alkaline reaction fairly soon, and as the concentration of phosphate in the standard medium is small, the slight initial differences in acidity produced by the different phosphates are soon obliterated.   With higher concentrations of phosphate (e. g. 1 per cent.) a marked difference appears according as the neutral or acid forms are used. (One can formulate the rule that whereas increase of neutral phosphate causes increased sporulation and reduction of aerial mycelium, increase of phosphate in the acid form increases mycelium formation and strongly reduces sporulation)

Certain features of the physiological effect of phosphate will be dealt with in connexion with the discussion of the nitrogenous constituent.

II.   *The Nitrogenous Constituent.*

The nitrogenous constituent chiefly experimented with has been asparagin.   Other compounds have been tested to a less degree—viz. potassium nitrate, various salts of ammonia, and peptone.

The effect on the growth-form of the fungus of variation in the nitrogenous constituent will only be dealt with in part under the present heading. It will be shown later that, over and above the actual concentration of nitrogenous compound, the ratio of the carbon to the nitrogen compound is a factor of considerable importance in influencing the form of growth. Certain aspects must therefore be left over until the effect of the carbon compound is dealt with.

The effect of asparagin concentration on colony growth is very similar to that described in connexion with simple dilution or concentration of the whole medium.   Reduction of the asparagin content of the medium eliminates the staling effect in all the strains, the difference between a more strongly and a more weakly staling strain being that dilution has to be carried farther in the former case in order to produce the non-staling type of growth.   With increased asparagin content the staled type of growth appears in all cases.

With increasing asparagin content one finds increasing evolution of ammonia from the cultures.   This effect is easily demonstrable in the case of cultures on the standard medium; when the asparagin concentration is increased to 0.5 per cent., ammonia can easily be smelt, and at 1 per cent. asparagin the cultures may be described as reeking of ammonia.

The effect of varying the asparagin content on the development of aerial mycelium is somewhat slight.   With a strain which has aerial mycelium on the standard medium, reduction in the amount of asparagin

ultimately causes the disappearance of aerial mycelium, but the effect is less marked than in the case of mere dilution of the medium. Increase in the amount of asparagin in all the strains (except the pionnotal) leads to increase in amount of aerial mycelium and diminution, and finally inhibition, of sporulation. The aerial mycelium formed under these conditions has, however, an abnormal appearance, appearing somewhat sodden and with a greyish colour. The spores also have a very abnormal stunted appearance. There is no doubt that such colonies are growing under very unhealthy conditions, and are in fact suffering from ammonia poisoning.

Perhaps the most striking effect of varied asparagin content is that on the colour formed in the medium. All the strains when grown on the standard medium are colourless—that is, apart from the colour of the spore masses and from a slight pink coloration in the mycelium when the cultures are grown in the light. When, however, the asparagin content is reduced sufficiently, a further colour effect appears, viz. in the medium itself. The intensity and tone of this colour under identical conditions vary from strain to strain. The manner in which the incidence of this colour is dependent upon asparagin concentration is best illustrated by a particular example.

The organism was strain A. The basal medium contained 0·125 per cent. K_3PO_4, 0·075 per cent. $MgSO_4$, 2 per cent. potato starch. The following media were set up, with asparagin concentration as given :

I,	none	
II,	0·005	per cent.
III,	0·01	„ „
IV,	0·02	„ „
V,	0·05	„ „
VI,	0·1	„ „
VII,	0·2	„ „
VIII,	0·3	„ „
IX,	0·4	„ „
X,	0·5	„ „

The colonies were grown at laboratory temperature, which varied between 15° and 20° C. Colour first appeared in II on the ninth day ; then on the tenth day in I and III ; in IV on the twelfth day ; in V on the fifteenth day. A description of the colour appearances presented after twenty-one days' growth (by which time the colonies on media I–VII had covered the whole plate) is as follows :

I, moderate yellow colour throughout the whole colony.

II, strong „ „ „ „ „ „

III, similar to II.

IV, only moderate yellow to radius 1·5 cm., then strong yellow beyond up to edge of colony.

V, almost colourless to radius 2·0 cm., then moderate yellow up to edge of colony.

VI, colourless to radius 4·0 cm., then feeble yellow zone beyond.

The remaining colonies were devoid of colour.

The effect of increasing the asparagin concentration from minimal amount may thus be summarized:

Increase of asparagin at first increases the intensity of colour, probably by increasing the amount of growth possible; on further increase, the intensity of colour diminishes, thinning out at first at the centre of the colony. By and by the centre of the colony fails to develop colour, and, with progressive increases of asparagin, the coloured zone retracts to the outside of the colony and finally disappears.

It is interesting to note that colour is frequently accentuated opposite an accidental contamination, e. g. a *Penicillium*. When the concentration of asparagin is such that the *Fusarium* just fails to produce colour, the presence of a *Penicillium* colony will cause it to show colour where it comes against the contamination.

The majority of the strains produce a yellow colour as in the case of the strain A. The colours produced by the remainder are blue, violet, or pink. The degree of dilution of the asparagin constituent necessary for colour production varies somewhat from strain to strain. Thus a strain A_1, a saltant from strain A, differs from the latter, among other things, in remaining colourless under conditions in which A is strongly coloured. Further reduction of the asparagin content nevertheless causes colour production in strain A also, though not to the degree shown by strain A at its optimal concentration.

Temperature affects colour formation inasmuch as higher temperature within limits intensifies the colour and causes it to develop sooner. With a strain which forms a clear yellow colour at 15° C., the colour formed at 25° C. may be brown or almost black, especially round about the centre of the colony. Temperature does not appreciably influence the degree of dilution of the N-constituent at which colour formation begins. Thus a medium suitable for colour formation will give colours at high or low temperature, whereas a medium in which no colour is formed at a low temperature will form none if the temperature is raised.

Light or darkness has not been observed to produce any effect on colour formation in the medium.

With *potassium nitrate* as the source of nitrogen, the same effects in general are produced on growth-form as with asparagin, with this difference, that with increased concentration of potassium nitrate the staling effects are not so intense and there are not the marked deteriorating or poisoning effects obtained with high asparagin content. When the potassium nitrate content is reduced sufficiently far, the same colour effects are obtained as

with reduced asparagin. For this purpose a concentration of 0·02 per cent. KNO_3 is approximately equivalent to 0·03–0·05 per cent. of asparagin.

Peptone in its effect on growth-form is equivalent to a much smaller concentration of asparagin. With standard medium, the growth-form obtained with 0·2 per cent. peptone is comparable with that given by 0·02 per cent. asparagin. At moderately high concentration it is obvious that the higher carbon value of peptone would come in as an additional factor affecting growth-form.

When the asparagin in the standard medium is replaced by an equal weight of *ammonium chloride,* a colony is obtained with a zone of marginal aerial mycelium and central sporing region. Increase of the ammonium chloride reduces both the amount of aerial mycelium and spore formation, the former disappearing when the concentration of ammonium chloride reaches 1 per cent., and both when the concentration reaches 2 per cent. There is increasing tendency to staling* with increasing concentration of ammonium chloride, and all the cultures become acid.

Reduction of the ammonium chloride content produces much the same effects as in the case of asparagin; here, again, colour formation begins when the concentration is sufficiently reduced. In this respect ammonium chloride is approximately equivalent, weight for weight, to asparagin.

Asparagin in relation to phosphate. Asparagin and neutral phosphate act in some respects antagonistically to each other. Thus the effect of increased staling got by increasing the concentration of asparagin can, within limits, be removed by a sufficient increase in the concentration of neutral phosphate. The following figures give the diameters of colonies of strain A reached in fourteen days on a medium in which the asparagin and phosphate were varied as indicated.

Asparagin.	*Phosphate.*		
	0·125 per cent.	0·5 per cent.	2 per cent.
0·05 per cent.	9·7	9·8	9·4
0·2 „ „	5·6	5·7	8·6

With the two lower concentrations of phosphate, quadrupling the asparagin content brings on distinct staling; at the higher concentration of phosphate, this effect is practically obliterated.

It has been shown above that the factor inducing colour formation is a reduced concentration of the N-constituent, also that the same colour effects are produced in a minor degree when the phosphate is reduced to minimal concentration. When a medium is such that colour is formed, an increase either of N-constituent or of phosphate will cause diminution of colour, but the N-constituent is much more potent than the phosphate in this respect.

III. *The Carbonaceous Constituent.*

In the standard medium two sources of carbon are used, viz. glucose and asparagin.   That the latter is a feeble source of carbon is shown by the thin colonies produced when glucose is omitted.

The standard medium has an excess of N-constituent in relation to the amount of carbon present.   This is seen from the fact that a certain amount of the nitrogen is evolved as ammonia.   On increasing the amount of carbon, the immediate effect is to increase the density of growth of the colony, and thus the effect of increasing the carbon content of the medium might be expected to be similar to that produced by simple concentration of the medium.   There is, however, another effect, viz. that increase of the carbon constituent counteracts the development of alkalinity arising from such a nitrogenous constituent as asparagin.   Increase in concentration of glucose in so far as it means increased amount of growth means increased staling ; in so far as it antagonizes the staling products derived from the asparagin it reduces staling.   The net effect on the rate of growth of the colony consequent on a change in concentration of the carbon constituent may in particular cases be quite small, e. g. with such a weakly staling form as strain A.   The salient features of the curve describing colony size in terms of glucose concentration are two.   (1) When the glucose concentration is reduced to minimal values there is a tendency to staling in all forms.   As the colonies formed are very thin. and therefore the total amount of staling substances is small, the effect is not very pronounced, but such as it is, it is obviously due to the failure to counteract the staling products derived from the asparagin.   (2) Whether the particular strain shows staling effects weakly or strongly on the standard medium, increase in the amount of glucose, if carried far enough, gives the non-staling growth in all cases.

The curve of growth (linear) obtained by varying the glucose constituent is thus in its main features the reciprocal of that obtained by varying the asparagin.   The magnitude of the effects produced is, however, much smaller in the former case.

In a medium containing an excessive amount of ammonium chloride as the source of nitrogen, the effect of variation in the amount of glucose on colony diameter is the opposite of that just described.   The reason for this is simple.   Increase in glucose concentration means increased formation of mycelium, and therefore more ammonia assimilated, and finally more hydrochloric acid set free.   Staling, due to excessive liberation of acid, thus sets in.

As regards amount of sporulation, the effect of varying the glucose constituent is very similar to that shown on varying the total concentration of the medium.

Perhaps the most striking point brought out in connexion with the influence of carbon constituent upon the growth form of the fungus is that all the forms of carbohydrate tested are not equivalent. Glucose, saccharose, and maltose, in equal amounts, are practically equivalent, but starch is not equivalent, weight for weight, to any of these simpler carbohydrates. All the *Fusarium* strains readily decompose the starch in their neighbourhood, nevertheless a given concentration of starch, though convertible theoretically into the same concentration of glucose, is not equal in its effects to the latter. As regards the amount of mycelium produced, a given concentration of starch is equivalent to a much smaller one of glucose. The difference, however, is not merely quantitative. Whereas in general increase in the glucose constituent leads to an increase in mycelial growth with, in the higher concentrations, suppression of sporulation, increase in the starch content produces somewhat insignificant increase in mycelium formation but a striking increase in the amount of sporulation.

A few illustrations will serve to show the kind of result obtained.

Strain A was grown on a medium containing: Asparagin, 0·4 per cent. ; K_2PO_4, 0·125 per cent. : $MgSO_4$, 0·075 per cent., together with the following percentages of glucose and starch :

	I	II	III	IV	V	VI	VII
Glucose	0·2	0·4	0·2	0·7	0·2	1·2	0·2
Starch	0	0	0·2	0	0·5	0	1·0

It will be noticed that medium II contains the same amount of carbohydrate as III, only that the former contains it all as glucose, and the latter partly as glucose and partly as starch ; also that the same applies to the pairs of media IV and V, and VI and VII.

When the resultant colonies were arranged in order of increasing mycelium formation, the series was as follows :

$$I < III < V < II < VII < IV < VI.$$

As the basal medium had a somewhat high concentration of asparagin (twice that of the standard medium) there was strong staling on medium I. There was much less staling on medium VI, and the other media arranged themselves in this respect in the same order as that just given. Thus the greater effect of glucose in producing mycelial growth as compared with starch is paralleled by a greater potency in overcoming the staling effect introduced by an overdose of asparagin. In respect of both these growth features, medium II lies between V and VII, i.e. 0·4 per cent. glucose in its effect on growth-form is intermediate between 0·2 per cent. glucose + 0·5 per cent. starch, and 0·2 per cent. glucose + 1 per cent. starch. Hence one may say that 0·2 per cent. glucose is equivalent to something between 0·5 per cent. and 1 per cent. starch.

A simultaneous experiment with strain C 3 gave the series as follows :

$$I < III = V < II = VII < IV < VI$$

which points to a similar conclusion to that just stated.

The different effects of starch and glucose on sporulation have been repeatedly confirmed.   The following data will serve to illustrate the point :   In a series of media with the usual basal mineral concentration, the combinations of carbohydrate and asparagin were varied as follows :

Glucose	0·2 per cent.			
,,	0·6 ,,	Asparagin	0·02 per cent.
,,	2 ,,	,,	0·05 ,,
Starch	0·2 ,,	,,	0·2 ,,
,,	0·6 ,,		
,,	2 ,,		

Six of the *Fusarium* strains were grown on these media and the varying growth-forms compared.   The following data give the main outlines of the sporulation features observed in the case of strain A :

Asparagin 0·02 per cent.

Glucose	0·2 per cent.,	scattered but abundant sporodochia to radius 3·5 cm.
,,	2 ,,	a few irregularly scattered sporodochia.
Starch	0·2 ,,	as in corresponding glucose medium, but denser sporulation.
,,	2 ,,	spores covering same area as in the last, but definitely denser.

Asparagin 0·2 per cent.

Glucose	0·2 per cent.,	strong sporulation in rings to radius 4·0 cm.
,,	2 ,,	interrupted ring of spores at radius 4·0 cm. ; apart from this a few scattered sporodochia.
Starch	0·2 ,,	spores in zones to nearly edge of plate (5·5 cm.).
,,	2 ,,	spores in continuous layer, nearly to edge of plate ; sporing here most intense of all.

The other media were intermediate in their respective series.   The general result is that though the increase in starch did not always increase the area of the sporulating region it always increased the intensity ; on the other hand, increase of glucose had the reverse effect.   Correlated with these results, none of the starch series showed any aerial mycelium, while the cultures on the medium with highest glucose and highest asparagin were almost completely covered with aerial mycelium.

An exactly similar type of result was obtained with strain C 3, another sporodochial strain.   With poorly sporing mycelial strains, results different

in detail but pointing to the same conclusion were obtained. Here the highest starch concentration did produce aerial mycelium formation, but nothing compared with that produced by an equal amount of glucose, and in the case of some of these feebly sporing strains it was only in the starch series of media that any visible sporulation was obtained at all.

Considerable attention has been paid to this difference in the behaviour of glucose and starch in the nutrition of these fungi. The results above outlined have been found to hold for all the strains tested and for media in which the glucose, starch, and asparagin contents have been widely varied, as also for media in which the source of nitrogen was potassium nitrate or ammonium chloride. In all cases it has been found that a given increase in the starch content of the medium is much less potent in changing the growth-form than an equal concentration of glucose. That there should be a difference might be expected *a priori*, since in the case where glucose is used the maximum concentration is present at the beginning, whereas when the same amount of carbohydrate is present as starch the active concentration of diffusible sugar at any time might be small. But it is noteworthy that the whole of the starch in the medium may disappear and nevertheless the resultant amount of mycelium is obviously much less than in the case with the glucose medium. The question arises as to what becomes of the carbohydrate in the case of the starch medium, as it appears to be insufficiently represented at the finish by the carbonaceous material of the mycelium. Respiration differences thus seem to be indicated, and a physiological examination along these lines is being made.

In a medium containing both glucose and starch the former will outweigh the latter in its effect if the two carbohydrates are present in anything like equal proportions. Similarly the addition of a moderate amount of starch to a glucose medium only slightly affects the growth-form, whereas the addition of the same amount of glucose to the corresponding starch medium is much more likely to produce a distinct effect on the form of the colony.

Carbohydrates as affecting the Colour produced by the Fungus.

It was shown above that the necessary condition for colour formation in the medium in the case of these *Fusarium* strains was to reduce the concentration of the nitrogenous constituent to a sufficiently low limit. This low limit depends, however, on the concentration of the carbohydrate present—in other words, the colour-producing factor is not so much the low concentration of N-constituent as a high C/N ratio in the medium. This will be illustrated by a particular experiment with strain A.

In a medium containing the usual amount of potassium phosphate and magnesium sulphate, the C-constituent (starch) and the N-constituent

(asparagin) were varied against each other according to the following scheme :

Asparagin.	*Starch.*			
	0·2 per cent.	0·5 per cent.	1 per cent.	2 per cent.
0·02 per cent.	A α	A β	A γ	A δ
0·05 ,,	B α	B β	B γ	B δ
0·1 ,,	C α	C β	C γ	C δ
0·2 ,,	D α	D β	D γ	D δ
0·5 ,,	E α	E β	E γ	E δ
1 ,,	F α	F β	F γ	F δ

Colour first appeared in six days in medium A δ; then in succession at one to two days' interval in B δ, A γ, B γ, A β, and so on. After forty days the colour appearances presented were as follows :

δ *series.*   A, strong yellow throughout.
       B, reduced yellow at centre, but strong beyond.
       C, colourless to radius 3·5 ; moderate yellow beyond.
       D, colourless except for narrow yellow ring at edge of plate.
       E and F, no yellow.
γ *series.*   A, slight yellow to radius 3·8, strong beyond.
       B, similar to last, but less intense colour.
       C, colourless except for marginal ring 1 cm. wide.
       D, trace of yellow at edge, otherwise none.
       E and F, none.
β *series.*   A, colourless to radius 4·0, then slight yellow.
       B, colourless except for thin marginal zone.
       C, D, E, F, no colour.
α *series.*   No colour in any case.

It is obvious from these results that the condition for colour formation is a sufficiently high C/N ratio. The same kind of result was obtained when the source of nitrogen was potassium nitrate or ammonium chloride.

Experiments in which glucose was substituted for starch led to an unexpected result, for when the glucose concentration was increased, intensity of colour increased up to a point and then again diminished—that is, that for a given concentration of asparagin there was an optimum concentration of glucose, beyond which colour formation again diminished. With a similar concentration of starch there was no evidence of an optimum concentration having been reached.

When in a medium containing the carbohydrate as starch the C/N ratio is progressively reduced, the colour effects are lessened in the regular manner above described, i. e. the colour becomes paler in the centre of the plate and eventually vanishes at the edge of the plate. When a similar test is made with glucose as carbohydrate, the same series of appearances are in some case obtained, but not always. The colour in some cases fades from the margin inwards or the last traces may be shown half-way to the

edge of the colony. These varied effects are probably due to the existence of an optimum concentration for colour formation in the case of glucose.

Partly on account of these less regular appearances and partly on account of the aerial mycelium which tends to be formed when glucose is used as source of carbon, and which interferes to some extent with observation of the colour, it is preferable to use starch as the chief source of carbon for colour study. A common method for testing the colour reactions of *Fusarium* is to add an excess of carbohydrate, such as 5 per cent. sugar, to a natural decoction, such as potato agar. This acts by increasing the C/N ration, but it is obvious in the light of the above that it is a method which is susceptible of improvement.

The standard medium that has been used in the study of the colour reactions of the present group of forms is as follows:

Glucose	2	grm.
Potato starch	10	,,
Asparagin	0·2	,,
K₃PO₄	1·25	,,
MgSO₄	0·75	,,
Agar	15	,,
Water	1 litre.	

The colour effects produced on this medium are much more defined and easy of description than on such a medium as potato agar plus 5 per cent. sugar. This medium is also suitable for sporulation in the case of all but the most feebly sporing strains.

G. MISCELLANEOUS CULTURAL FEATURES.

Certain observations of a miscellaneous character will now be dealt with.

Zoning. A kind of zoning may be shown in the colour effects, as has been already stated, but the most striking zonal effect shown by some of these *Fusarium* strains is in the arrangement of the spore masses. Some strains, e. g. D, form on the standard medium one strong zone of spores. This obviously has no relation to the periodicity of light. Others form definite series of rings corresponding to the alternation of day and night, e. g. strain A. The latter type of zonation is only shown by those strains which form their spores in sporodochia, and which are growing in the unstaled manner. This condition, though apparently necessary, is not sufficient to ensure zoning, as some non-staling sporodochial strains show no zonal arrangement.

That the day-by-day formation of spore rings should be associated with the unstaled type of growth is understandable. If growth is unstaled, it means that the growing edge from day to day is under the same or approximately the same physiological condition, and therefore will react uniformly from day to day. Once staling begins, the growing edge is in a different physiological state from what it was before staling began, and

therefore will cease to react as formerly. In particular, the incidence of staling means in general that a zone of somewhat permanent mycelium will form at the edge and no further day-to-day zones are possible. A marginal zone of spores may subsequently form there, but this will no longer have any relation to the alternation of day and night. This correlation of zoning with the unstaled type of growth has been found to apply to the well-known zonal effect shown by *Monilia fructigena.*

The conditions necessary for daily zonation in response to light may be summarized as follows: (1) The fungus should be affected in its sporing capacity by light; this means in general an increased tendency to sporulation in the light. (2) The region of mycelium which is sensitive to light should from day to day be maintained in approximately the same physiological state. This means the non-staling type of growth. (3) The medium should not be of a concentration to allow of sporulation being so intense that the successive zones fuse together, with the result that any zonal arrangement present is hidden under a continuous layer of spores.

As these *Fusarium* strains in general sporulate most intensely in the neighbourhood of the centre of the colony, one finds in a zoning strain that the central region of the colony is covered by a more or less continuous mass of spores; beyond this discrete zones are formed.

The tendency to form zones is a function of the particular strain, and will in general have some systematic value. Some of the strains (even of the sporodochial type) have never been seen to form zones, whereas a zoning type will show a general tendency in that direction over a range of media, though the clearness with which the effect is shown varies sharply with the nature of the medium.

Colour. The conditions favouring colour formation have been described above. Some further particulars, chiefly relating to the pigments themselves, may be mentioned here. In consequence of the high C/N ratio in the colour-producing medium, the latter develops a slightly acid reaction as a result of the fungal growth. Whether this reaction must always be present in order that colour may arise is not known, but it has always been found to be present in such cases. Different strains appear to produce different degrees of acidity on the same medium (a medium with high C/N ratio), and it may be possible to correlate the amount of colour formation with this acid factor.

The yellow pigment formed by most of the strains is present in solution in granules encrusting the fungal hyphae. A proportion at least of these granules stain red with iodine, and they probably consist of glycogen. If this is so, it indicates an interesting enzymatic reaction. Under the conditions present, viz. excess of starch, the latter is broken down to a simple carbohydrate, and some of the unused carbohydrate again synthesized to glycogen. From the occurrence of the glycogen *outside* the hyphae, it is suggested

that this synthesis is being carried out by the glycogenase enzyme in the medium, i. e. outside the hyphae. The pink, blue, violet, &c., colours are found diffused in the medium. All these colours are to some extent indicators. Thus the addition of dilute sulphuric acid to a yellow plate causes the almost immediate disappearance of the colour. The blue coloration under the same conditions is changed to pink.

Sclerotia. The usual teleological 'explanation' of sclerotia is that they are the means of carrying the fungus over unfavourable times, and that they are called forth by such conditions as exhaustion of food, or by staling effects in the medium. As far as concerns these *Fusarium* strains such an explanation is not in accordance with the facts. The best development of sclerotia is found in that group of strains which is characterized by a tendency to form persistent mycelium, such as strain D. Strains which have a strongly sporulating capacity—such as the pionnotal strains—have conversely the feeblest tendency towards sclerotium formation. Similarly none of the strains forms sclerotia under conditions in which it sporulates freely. As sporulation is correlated with autolysis of mycelium and sclerotium formation connotes accumulation of mycelium, it is obvious that the two are in the main antithetic phenomena.

To consider the case of such a strain as D, no sclerotia are formed under conditions of comparatively feeble nutrition, e. g. on the standard medium or any dilutions of it. When in the standard medium the glucose and asparagin concentrations are raised to 2 per cent., a strong weft of mycelium and a few small sclerotia are formed. This medium becomes too stale through alkali formation to allow of full development of sclerotia. To fit the medium for this purpose it is necessary to reduce the staling factor by diminishing the asparagin. When therefore in the standard medium the glucose and asparagin concentrations are raised to 2 per cent. and 0·4 per cent. respectively, a medium is obtained which allows of strong mycelial growth, and which becomes stale comparatively slowly; on such a medium sclerotia are formed freely. A medium which is similarly suitable is Richards's solution. (The high concentration of sugar (5 per cent.) ensures strong development of mycelium, and the presence of potassium nitrate as the source of nitrogen, together with that of acid phosphate, ensures that growth is not stopped by too early staling. The conditions for sclerotial formation are thus: (1) a rich medium, i. e. one with high carbohydrate content; (2) minimal staling conditions consistent with the strong growth required. It may well be that under such conditions a large proportion of the food is absorbed by the time the sclerotia are fully developed, but it is much more rational to consider that sclerotium formation is due, not to the poorness of the medium which remains, but to the richness of the medium at the start and to the conditions which allowed of its utilization.

It is interesting to note in this connexion that *Botrytis cinerea* and

Sclerotinia Libertiana, which characteristically produce sclerotia, are fungi showing very slight staling tendencies.

H. Summary.

1. The variations in growth of a number of *Fusarium* strains in response to changes in environmental factors have been examined. The factors to which attention has been mainly directed are the concentration and composition of the nutrient medium, and to a less extent certain physical factors such as light and temperature.

2. From a comparative study of the rates of colony growth it is shown that :

(*a*) All the strains have very approximately the same intrinsic growth-rates.

(*b*) They differ from each other as regards the degree of staling shown.

(*c*) Any strain may be made to grow in the staling or non-staling form by sufficiently concentrating or diluting the nutrient medium.

3. The effect of concentration of the nutrient on amount and distribution of sporulation and of aerial mycelium is described for a number of type strains. Increase of concentration up to a certain point produces increase of sporulation, but increase beyond this limit produces diminished sporulation, on account of the increased tendency to the formation of persistent aerial mycelium. For the majority of the strains the maximum intensity of sporulation is shown round about that concentration at which the transition , from the non-staling to the staling type of growth takes place.

4. The dependence of growth-form upon the nature and concentration of the most important constituents of the nutrient medium has been examined in detail. The following are the main results :

(*a*) Increase of phosphate in the form of the neutral salt increases sporulation and diminishes aerial mycelium formation, and vice versa. Acid phosphate produces the opposite effect.

(*b*) The nitrogenous constituent is chiefly responsible for the intensity of the staling reactions shown.

(*c*) Glucose and starch have different effects, both quantitatively and qualitatively, on growth-form. Increase in the concentration of glucose tends to produce greater development of aerial mycelium ; a similar increase in the concentration of starch results chiefly in more intense sporulation.

(*d*) Colour formation in the medium depends chiefly upon a high carbon : nitrogen ratio in the nutrient. A low concentration of phosphate is also within limits favourable to colour formation. A synthetic medium suitable for showing colour effects in these strains is given.

5. A number of observations are recorded on the phenomena of zonation and sclerotium formation.

The Past and Present Distribution of the Magnolieae.

BY

R. D'O. GOOD, B.A.

With one Figure in the Text.

I NTEREST in the subject of plant distribution has been revived consider-
ably within the last few years by the publication of the ' Age and Area '
hypothesis of Willis (1). Its postulation has been the cause of much con-
troversy, and, despite the wealth of argument with which it has been presented,
it is, in the opinion of many botanists, incapable of explaining the main
problem of plant distribution. This problem is, as Schimper has said (2),
the explanation of the differences between the various floras of the world
to-day.

Dr. Willis's hypothesis may be looked upon as the antithesis of those
theories which attribute the present distribution of plants mainly to the
continued effects of the changes which have occurred in the various external
factors to which plants have been exposed during their history. Such
theories may be said to have originated with the Theory of Climatic
Migrations put forward by Prof. E. Forbes in 1845 (3). They do not
specifically deny the conception of Age and Area, but tacitly assume that
its value as an explanation of distribution is very limited. Willis, on the
other hand, considers Age and Area to be of paramount importance, and
that the results of migrationary influences occupy a subordinate position.

The student of plant distribution to-day is thus faced with two antago-
nistic theories to explain the facts with which he deals, and it is necessary
to come to some conclusion as to their relative values. It is improbable
that one can be accepted to the rigid and total exclusion of the other, and so
the task becomes one of compromise. The methods adopted in this paper
will not appeal to the enthusiastic supporter of Age and Area, since the
plants selected for discussion are some of those which afford the most
obvious and often-cited exceptions to the hypothesis. This one case has
simply been taken as affording the best means of setting out and illustrating
the various aspects of the past and present which bear upon the subject
under discussion.

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At the same time it is necessary to point out that the hypothesis of Age and Area is not quite comparable with that of Climatic Migrations. The former describes a result rather than a cause: it assigns no driving force to its fulfilment other than the suggested absence of certain disturbing factors, and these are the very factors which to the best of our knowledge have been present. The migrationary hypothesis, on the contrary, suggests that the distributional phenomena in plants are due to and are directly caused by those various environmental changes which are generally believed to have taken place, and for which there is abundant evidence.

In all distributional theories the question of plant dispersal and the value of dispersal mechanisms bulks largely. No definite conclusions have as yet been reached as to the relative values of these mechanisms, or even whether the possession of such has any very great bearing upon the distributional potentialities of their possessors. One general statement can, however, be made. It is that dispersal mechanisms are only the means by which plants actually accomplish their movements from one generation to another. Not until such movements are *permitted* by external factors can such movements be *accomplished* by dispersal mechanisms.

Forbes's original suggestions were published in a paper read to the British Association in 1845 (3) under the title 'On the Distribution of Endemic Plants, more especially those of the British Isles, considered with regard to Geological Changes '. The subject-matter of this paper was much enlarged and presented in greater detail during the following year in a Memoir of the Geological Survey (4) entitled ' On the Connexion between the Distribution of the existing Fauna and Flora of the British Isles, and the Geological Changes which have affected these Areas, especially during the Epoch of the Northern Drift '. In this paper the author takes for granted the existence of specific centres of creation. He shows that isolated areas may be populated in three ways—by special creation within the area, by transport to it, and by migration into it before isolation—and he expresses the view that the first two methods are inadequate, while the third is of greatest importance. The remainder of the paper is best summarized in the author's own words. He says: ' the specific identity to any extent of the flora and fauna of one area with those of another depends on both areas forming or having formed part of the same specific centre, or on their having derived their animal and vegetable population by transmission, through migration over continuous or closely contiguous land, aided in the case of alpine floras by transportation on floating masses of ice '.

This work was extended and discussed by Darwin (5) in the ' Origin ' and by other contemporary writers (6, 7). More recently Prain (8), in his monograph on *Pedicularis*, gives a very clear and concise account of the hypothesis of Climatic Migrations, which he considers is supported by the results of his own work.

Systematic Notes.

Before passing to the detailed distribution of the Magnolieae, some explanation of the methods of presentation is necessary. For the sake of unity and convenience the terms of systematic botany are used, but merely as general terms and in no way affording definite systematic values. The term species is used, for example, to denote units of approximately equal value, from which the data of distribution can be drawn. No attempt is made to use the word in a critical sense. The same remarks apply to the term genus. This explanation is the more essential since there is no up-to-date monograph of the Magnolieae, and some considerable difficulty has been experienced in deciding upon the total number of species to be considered. The method finally adopted has been to take into consideration those species which have apparently retained their autonomy since their original descriptions. The Kew Index has been the basis for this procedure. Such a method probably errs on the side of too many species, but the error is likely to be similar in proportion throughout the group, and in such work as this it is the relative numbers which are of greatest importance. Another great difficulty which is encountered is that in very many of the species the distribution is incompletely known. One or two are, in fact, known only as single plants, while in other cases species long known only from single plants have been discovered in plenty in newly explored regions. Another aspect of the same difficulty is that newly described species are, by the nature of the case, known from very circumscribed areas. This is particularly felt in the case of the Chinese species, of which many have been described in the last few years. There is no remedy for these troubles if the work is to be done, and the data must be used as they become available. Due allowance for this must be made when theoretical considerations are discussed.

Different writers have classified the Magnoliaceae in many different ways, but in all the central group is the sub-family of the Magnolieae. In the present paper this group is considered as containing some 130 species divisible into two very unequal parts. The smaller contains but one species, *Liriodendron tulipifera*, which differs from all the rest in the shape of its leaves, the extrorse anthers and the winged carpels. All the other species have entire leaves, introrse anthers, and winged carpels. In all classifications the former plant forms the monotypic genus *Liriodendron*. This plant is not further considered here since the account of it is similar to and epitomizes that of the rest of the Magnolieae. The varying separations of the remaining species into genera have been numerous, ranging from that of Spach (9), who included ten genera, to that of Engler and Prantl (10), who make three. The best known is perhaps that of Bentham and Hooker (11), who recognize four genera, *Magnolia*, *Manglietia*, *Michelia*, and *Talauma*. In this scheme,

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however, the generic distinctions are by no means equivalent, since *Manglietia* only differs from *Magnolia* in the number of ovules in each carpel. In *Michelia*, on the other hand, the number may vary from two to five or even eight in each carpel. Engler and Prantl's system avoids this anomaly and has been followed here. As has been stated above, this is done purely on the score of convenience, but at the same time probably represents a fair critical division. Here, therefore, the Magnolieae are treated as composed of three groups or genera (*Liriodendron* being omitted). These are characterized as follows:

MAGNOLIA. Fruits without a gynophore, carpels closely packed, not separating at maturity. 63 spp.

MICHELIA. Fruits with a gynophore, carpels widely spaced, not separating at maturity. 25 spp.

TALAUMA. Fruits without a gynophore, carpels closely packed, falling free at maturity. 40 spp.

Manglietia of Bentham and Hooker is included in *Magnolia*, but is considered as far as possible as a separate sub-group characterized by having more than two ovules in the carpel. These various distinctions between the genera are not absolute. For example, one or two of the Magnolias (*M. Griffithii* and *M. Pealii*) show slight gynophore, while one or two Talaumas have a very slight wing upon the carpels. The fruit in certain Magnolias tends to be elongated.

DISTRIBUTION OF THE MAGNOLIEAE.

The present distribution of the Magnolieae as a whole covers two widely separated areas, one in the New World and one in the Old World. In the former, fewer species are found over a larger area than is the case in the latter, but the average areas of species in the two hemispheres are not very different. In each case the centre of distribution is towards the south-east corner of the northern land mass, i. e. in the south-east United States and in Indo-China respectively. These centres (from the point of view of species population) are also towards the northern distribution limit. Extending from these centres species are found in the western hemisphere, southward throughout Central America and the West Indies into South America, and in the eastern hemisphere through the Malayan Peninsula over nearly the whole of the Malayan Archipelago. In the New World two isolated species are found as far south as south-east Brazil, but in the Old World the southern limit is in the latitude of Java. The northern limits are respectively the great lakes of North America and Japan. The total number of species in America is about 24, representing two genera; in Asia about 104 species, representing three genera. *Michelia* and the *Manglietia* section of *Magnolia* are found only among the latter. The most thickly populated

portion in either area is the great mountainous region of Eastern India and Western China. Representatives of the group are found at all altitudes from sea-level up to 12,000 ft., but the great majority inhabit the higher levels. No really ' wide ' species are found, and nearly all are narrowly endemic. Leaving out of consideration, for the moment, the two Brazilian species there is a considerable similarity between the distribution in the two hemispheres. In each there is a northerly continental thickly populated area, and a southern more scattered insular area.

If the region of distribution is artificially divided up geographically the distribution of species is somewhat as follows :

North America .	. . 8	China–Indo-China	. . 35
Central America	. . 7	Japan	. 7
West Indies .	. 7	Malay Peninsula .	. . 8
South America .	2		——
Malay Archipelago .	. 34	Total .	. . 128
Himalaya–Burma .	. 20		

Distribution of Magnolia.

Magnolia, the largest genus, is also the most characteristically temperate. In the west it affords all the indigenous species in North America, and in the east it is the only genus native to Japan. The North American group (25) contains eight species, and Japan has seven including *M. compressa*, Max., in the Liukiu Is. and Formosa. Of these Japanese species *M. hypoleuca*, S. and Z., is also found in China, and *M. parviflora*, S. and Z., has a Chinese variety, while *M. Kobus*, DC., is indigenous also in the island of Quelpart, Korea, but not on the Asiatic continent. Returning to the New World there are two species in Mexico and one in Guatemala. The West Indian species comprise one in Haiti, one in Cuba, and two in Porto Rico.

Our knowledge of the continental Asiatic species has been much increased by the recent exploration of Western China (12), no less than fifteen species having been described from Yunnan, Schezuan, and Hupeh since the beginning of the present century. Two of these, *M. mollicomata*, W. W. Sm., and *M. tsarongensis*, W. W. Sm., extend into Tibet. These two bring the total of Chinese species, including the two mentioned above as being also Japanese, to twenty-two. One of these, *M. Duclouxii*, F. and G., is a *Manglietia*. It is interesting to note that none of them extend into the Himalayan region.

In the Himalayas and in Burma there are seven species including three members of the section *Manglietia—M. insignis*, Bl., *M. Caveana*, Hk. f. and Th., and *M. Hookeri*, Cub. and W. W. Sm. The Himalayan species include *M. Campbellii*, Hk. f. and Th., which will be referred to again later.

As a southern extension from the Chinese group there are three species,

M. Baillonii, Pierre, *M. Duperreana,* Pierre, and *M. Balansae,* A.DC., in Cochin-China. Similarly southwards from Burma there are four species in the Malayan Peninsula. Three of these are Manglietias, the fourth being *M. Maingayi,* King; two of the former extend farther south—*M. Sebassae,* Miq., into Sumatra, and *M. glauca,* Bl., over most of the Malayan Archipelago. It is also said to occur in the neighbourhood of Tonkin.

The species of the Malayan Archipelago include only one *Eu-Magnolia, M. javanica,* Kds. and Val., endemic in Java. The remaining four species are Manglietias, two occurring in Sumatra and two in Celebes.

The great majority of Asiatic Eu-Magnolias are found in the Himalayan–West China region, becoming increasingly fewer towards the south. Conversely with *Manglietia* the species (much fewer in absolute numbers) are predominantly equatorial and insular, and are rare on the continent.

Distribution of Talauma.

Talauma is the second genus which occurs in both eastern and western hemispheres. The total number of species is forty, nine being American and thirty-one Asiatic. Taking the former first, none are found in North America, the most northerly on the mainland being *T. mexicana,* G. Don., in Mexico. South of this are three other species, *T. sambuensis,* Pitt., in Panama, and *T. gloriensis,* Pitt., and *T. poasana,* Pitt., in Costa Rica. The West Indian Archipelago provides three species. Of these two are narrowly endemic, one in Cuba, *T. minor,* Urb., and one in New Granada, *T. Cespedezi,* T. and Pl. The third, *T. Plumieri,* DC., has a somewhat wider distribution, extending from Cuba in the west to Martinique in the east. The two Brazilian species, *T. dubia,* Eich., and *T. ovata,* St. Hil., complete the list of American Talaumas. These are found in South-east Brazil in the province of St. Paul at a latitude of 20 degrees South. *Talauma dubia* is a very little known plant said to have been collected by Pohl and Schott.

As in America, the Asiatic species of *Talauma* are also more characteristically equatorial than the Magnolias. Three species do, however, occur in the Himalaya. Apart from these the Asiatic northern limit of the genus is in the Philippines and in Cochin-China, the latter having one endemic species, *T. fistulosa,* F. and G. Another single isolated species is *T. andamanica,* King, in the Andamans. The remaining species of the genus are all found in the Malayan Peninsula and Archipelago. Three are confined to the Peninsula, while two others are found there but extend southwards, one, *T. elegans,* Miq., to Java, and one, *T. mutabilis,* Bl., to Java and Sumatra. This species also appears in south Indo-China. The residual species, some twenty in number, are scattered over the Malayan Archipelago; with one exception being confined to single islands or groups of islands. This exception is *T. Candollei,* Bl., which inhabits Java, Sumatra, N. Celebes, and British North Borneo. For the rest, there are three species in Sumatra,

four in Celebes, eight in the Philippines, two in Borneo, and two in New Guinea. The last form the south-east outposts of all the Asiatic Magnolieae.

Distribution of Michelia.

The genus *Michelia*, with twenty-six species, is confined to the eastern hemisphere. The great majority of the species are found on the Asiatic continent, the only exceptions being *M. cicatrisata*, Miq., in Sumatra, *M. platyphylla*, Merr., in the Philippines, and *M. celebica*, Koord., in Celebes. *M. Figo*, Spreng., is said to occur in Java, but is doubtfully indigenous; its real home being, apparently, South China and Indo-China. An extremely interesting species is *M. nilagirica*, Zenk., because it is the only member of the Magnolieae which is found wild on the Western Peninsula of India. It occurs in two varieties, one inhabiting the Nilgiri Hills and one in Ceylon.

The bulk of the genus falls into two groups—a western Himalaya–Burma group of nine species and a Chinese group of eight species. Of these latter, six have been described in recent years. The former group has a southern outlier, *M. manipurensis*, Watt, in Manipur, and the Chinese has a similar outlier, *M. baviensis*, F. and G., in Tonkin.

The genus includes three species of somewhat uncertain distribution. *M. Maudiae*, Dunn, is recorded from Hong-Kong only and is a doubtfully wild plant. The other two, *M. Champaca*, Linn., and *M. fuscata*, Bl., are extensively cultivated throughout Eastern Asia, and their real extent cannot be accurately determined. There is, however, evidence that *M. Champaca* is really wild in India and Burma, and that *M. fuscata* is a Chinese plant. The former particularly has undergone much at the hands of the systematists.

Having considered the distribution of the Magnolieae in some detail, the chief points may be summarized briefly.

The group is divided into New World and Old World areas. The centre of each is at or near the south-east corner of the northern land mass. In each case the majority of the species are continental and closely grouped, but in both there is an extension towards and across the equator. The North American species are paralleled by the Indo-China species, the Central American by those of the Malayan Peninsula and Sumatra, and the West Indies by those in the rest of the Malayan Archipelago. The occurrence of two Talaumas in Brazil is exceptional and isolated. There are no really widely distributed species, the widest being those extending from the Malayan Peninsula to various parts of the Archipelago. In fact it may be said that all the species are endemic. There are no species common to both hemispheres. The North American species are all narrowly endemic,

while the species of the Himalayas are usually distinct from the Burmese ones, and those of China are again distinct from these. The distribution of the species is generally closely correlated with that of the mountains, and the majority are found at considerable elevations. The most obvious exception to this is in the case of the North American kinds, but even here they are chiefly centred in the Appalachians. Except for the great divide of the Atlantic Ocean, and in the case of the Brazilian plants, no species is isolated from another by more than 200 miles of sea. From a systematic point of view the genera are very homogeneous, and specific differences are small. The Magnolieae form an excellent example of the similarity between certain elements of the floras of south-east North America and South-east Asia, first pointed out by Gray (13) and Hooker.

BIOLOGICAL NOTES.

The Magnolieae are all woody plants ranging in size from shrubs to tall and stately forest trees. The leaves are large and handsome, and in the more temperate species deciduous, although the majority are evergreen. A few, as *Magnolia acuminata*, Linn., are intermediate, the old leaves remaining on the tree until the following spring when the new leaves are put forth. One of the evergreen North American species, *M. grandiflora*, Linn., is perhaps the best known of all the Magnolieae, being extensively planted, often against the walls of houses, throughout Europe.

The flowers are large and handsome, especially in the Magnolias, and are white, red, or yellow in colour. Many are sweetly scented. Little is known of their pollination, but two species at least, *M. Yulan* and *M. grandiflora*, are said to be protogynous and entomophilous (14). The seeds are drupe-like and brightly coloured, and often remain hanging from the dehisced carpels by the persistent vascular strands of the funicle. They are acceptable to birds and certain animals, but there do not appear to be any definite records of the consistent dispersal of the plants by these agencies. The seeds quickly lose their power of germination.

One of the most striking members of the group is *M. Campbellii*, a native of the Himalayas at levels from 10,000 to 12,000 ft. It is a tall tree, up to 150 ft. in height, and when first described was comparatively common in the Sikkim Himalayas. Since then its numbers have greatly diminished owing to persistent felling for the sake of its timber. It is a deciduous species and flowers freely before leafing. The flowers are very large—as much as 14 in. across—pink inside and darker outside. The leaves are large, and the ripe fruits with their scarlet seeds make the tree a striking feature of the flora even after flowering is over. This species is illustrated in Hooker's 'Illustrations of Himalayan Plants' (15), and the same author refers to it in his journal (16) in the following words : 'Many species of *Magnolia* are found in Sikkim ; *M. Campbellii* of 10,000 ft. is the most

superb species known. In books on botanical geography the Magnolias are considered as most abounding in North America east of the Rocky Mountains, but it is a great mistake, the Indian mountains and islands being the centre of this natural order.' According to Grisebach (17) Magnolias and epiphytic orchids form a definite zone in the Sikkim at 9,400 ft., just below the bamboo zone and just above the laurels. This is the second highest zone of the tropical flora.

CLIMATIC RELATIONS (18).

Having considered the distribution of Magnolieae at some length it is interesting to see to what extent it is correlated with climatic conditions. A study of a rainfall map of the world at once reveals that the Magnolieae only occur in the regions of excessive precipitation. The Himalayan–Burma region, the Malay Archipelago, and Central America have all afforded many species. On the other hand certain other regions, such as Equatorial Africa, Madagascar, New Zealand, Chili, North Pacific America, and Northern Brazil, have no species. The possible explanation of this must be deferred to a later paragraph, the point of emphasis here being that apparently nowhere is there too much rain for these plants. Turning to the opposite extreme, it will be found that the Magnolieae occur nowhere where the total rainfall is below a certain comparatively high figure, somewhere round about 50 in. annually. This low limit is most nearly approached in the case of the North American species.

As regards the seasonal distribution of the rainfall there is considerable variation. In North America, Japan, Southern Malay Peninsula, and parts of the Archipelago the fall occurs at all seasons. In the remainder of Asia and Malay there is summer rain with a maximum at midsummer. In Central and South America the rainfall is intermediate in character. In Sikkim and the Khasias the total annual precipitation is over 160 in. The distribution of deciduous and evergreen species is roughly related to the seasonal variation in the rainfall. It is important to notice that when the amount of rain is least it is well spread over the year and nowhere is there a markedly dry season.

If the temperature is considered it will be seen that here again there is a bearing upon the distribution. The Magnolieae are not found where the average annual temperature is below 50° F. Here again the species of North America and Japan are the most tolerant, the figure, if these species are excluded, being between 65° and 70° F. The Talaumas alone are only distributed within the isotherm of 80° F. More important than the average annual temperatures are the isotherms for winter, January, and summer, July. As regards the former the species do not occur north of the isotherm 32° F. Certain of the North American deciduous species are the only ones which have to endure frost. In summer limits all the Magnolieae lie within the

isotherm of 64° F. The annual range of temperature ranges from 50° F. in Malay upwards. The highest annual maximum is reached in Burma—about 110° F.

If the temperature is taken alone the same features appear as in the case of rainfall, namely, the plants do not occupy all the possible areas. If, however, the rainfall and the temperature are considered together the result is much more striking. Under these conditions it is evident that the Magnolieae are found only in regions where there is a minimum rainfall of some 50 in. and a temperature within certain definite limits, not falling below a certain figure. Not only so, but with very few exceptions it is seen that the plants occupy the only areas in the world where this particular combination of conditions exists. The apparent exceptions are few but noteworthy, particularly so in the cases of North Brazil and North Pacific America, and these anomalies will be discussed later.

The result of the preceding paragraphs is to indicate that while the Magnolieae are found only in certain parts of the world, these parts are, with few exceptions, the only ones in which a given set of climatic conditions is to be found. Hence it is justifiable to suggest that these plants require such conditions for their perfect development, and are to be found only in such appropriate places. In this connexion it is interesting to note that, according to Wilson (19), *Magnolia grandiflora* thrives well in many parts of the southern hemisphere, but that Asiatic species are not successful in Australasia, except in a few New Zealand gardens.

THE FOSSIL RECORD.

In *Magnolia* the fossil record is fairly extensive, but most of the records are from fossil leaves. In addition, certain impressions have been identified as *Magnolia* fruits, and there are one or two records of seeds, including determinations by Clement Reid (20). These various records cannot all be accepted without comment, and it is necessary to come to some conclusion as to their value. It must be remembered that the only criterion lies in comparison with recent leaves.

None of the existing forms of *Magnolia* exhibit a type of leaf which is in any way characteristic, but rather show a very common biological type which is abundant in many different families. In a series of rubbings of a number of leaves, some of Magnolias and some from other families, it is practically impossible to detect any means by which the Magnolias can be distinguished from those of other families, especially Laurineae and Anonaceae.

This matter of leaf similarity has been discussed by several writers. Seward (21), in describing two fossil leaves from the Tertiary of Assam, points out that while they resemble certain fossils which have been identified as Magnolias, they equally resemble at least twenty other plants of very

varied affinity. Fritel (22) investigated the variation in leaf form of a recent species of *Magnolia*. Assuming a similar range of variation in fossil species, he concludes that a number of fossils, described under no less than nine generic names, may well be all different leaf forms of the species which he considers is certainly a *Magnolia*. In the same paper he mentions a so-called *Aralia* from the Miocene of south-west Siberia, and considers this is also a *Magnolia*. If this determination is correct, it enlarges the past distribution of the genus in a most interesting direction. Marty (23)
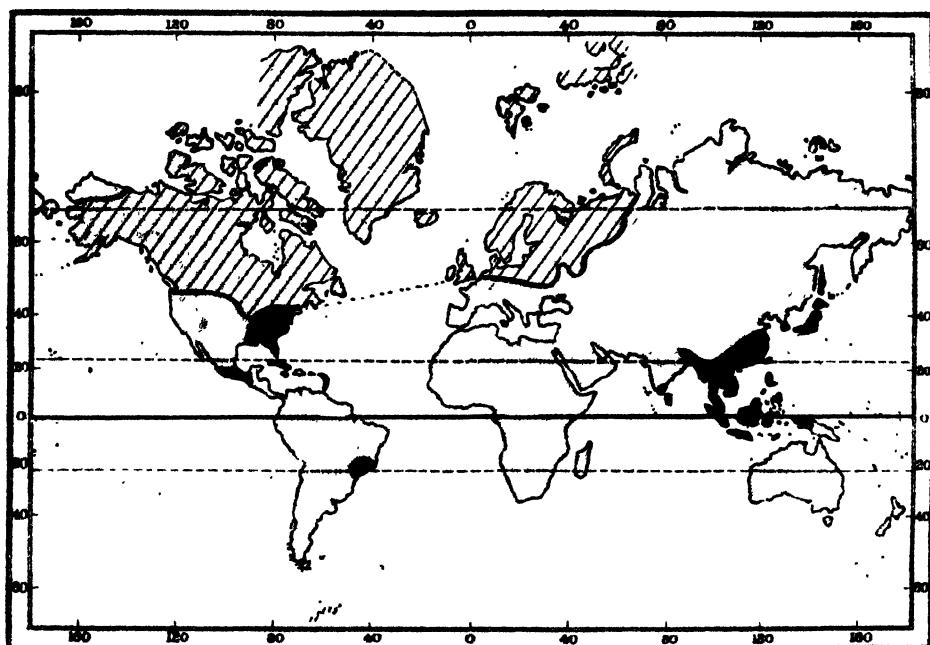
Map showing the present distribution of the Magnolieae (black areas) and the maximum extent of the Pleistocene ice (diagonally shaded areas). Note the overlapping of the two in eastern North America.

approaches the subject in a different way. He describes certain fossils from the Tertiary of Reval (Puy-de-Dôme), and points out the similarity to many recent forms, but by very careful and minute anatomical comparison he successively eliminates these plants until only *Magnolia* is left as a possible determination. At the same time he describes a fossil structure which bears a most striking resemblance to an unripened *Magnolia* fructification.

It is not possible to mention all the sources of the records of fossil Magnolias, but one or two particularly important works may be cited. Knowlton (24), in a memoir of the U.S. Geol. Survey, has catalogued all the American records in a most exhaustive manner. Berry (25), also,

has recently given a summary of the fossils, including the Old World records. Many arctic species are described in Heer's monumental ' Flora Fossilis Arctica ' (26). The following account is compiled from these and other papers.

The Cretaceous records show a wide distribution of the genus over the northern hemisphere. Fossils are known from a great many of the States of North America, and farther north there are records from Western Canada and Vancouver. In the Arctic regions many fossils have been found on the west coast of Greenland, in the vicinity of Disco Island. There are also Cretaceous records from Central Europe.

In the Eocene, again, fossil Magnolias are found over the wide areas in North America, including Western Canada and Alaska. The west coast of Greenland affords several species, and others have been found in Spitzbergen. In Asia there are records from the Island of Sakhalin. Continental European specimens are fairly numerous, and there are two determinations from the British Isles, from Alum Bay and the Isle of Sheppey.

Oligocene occurrences seem to be confined to Central Europe, but it must be remembered that America appears to lack plant-bearing beds of this age. In the Miocene fossils are known from beds in Europe and in North America. Finally, Pliocene fossils have been recorded from the Atlantic coastal plain of North America and from many of the countries in continental Europe.

This summary is given here without further comment. The difficulties of accurate determination have already been discussed. It is also in some cases impossible to decide the true age of the plant-bearing beds. It must also be borne in mind that the necessary conditions for preservation may have occurred only over narrow areas at different times, and thus the records at certain horizons may appear unduly scanty.

Despite these limitations, the total record is sufficient to illustrate the main important point, namely, that in the Cretaceous and Tertiary (or at least the earlier part of it) the genus *Magnolia* appears to have been very widely distributed in the northern hemisphere, extending to latitudes very much farther north than is now the case.

Only those fossils to which the name *Magnolia* has been definitely applied have been considered. There are also numerous others to which such names as *Magnoliastrum*, *Magnoliophyllum*, &c., have been given. As these names imply, they are merely suggestive of affinity, and are in no sense accurate determinations.

PALAEOGEOGRAPHY.

It is manifestly impossible in the course of a short paper to touch on more than a few of the appropriate points in the geological history

of the northern hemisphere. The most important of these points as they affect the Magnolieae are three in number : first, the past configuration of the land masses and the presence or absence of land bridges ; second, the distribution in time and space of the glacial epochs ; and, thirdly, the data and indications relating to past climatic conditions. Since there are no records of Magnolieae before the Cretaceous, previous periods need not be considered. Needless to say, much of the available material is purely supposititious.

Our knowledge concerning the past configurations of the land surfaces of the world has been collected and set out in a most complete manner by Arldt (27, 28). In particular he has included a series of maps showing the hypothetical land surface during each of the main geological epochs. These embody the combined opinions of the leading palaeogeographers, and much of the following matter has been taken from them.

In the Cretaceous times the land in the northern hemisphere appears to have formed a comparatively narrow continent extending completely round the globe. This continent had one extension northward, including the present North American archipelago and Greenland, and two extensions southwards, one down into South America and a smaller one covering the present area of Southern China. In the southern hemisphere was another almost circumpolar continent, discontinuous between Madagascar and Australia. The northern and southern continents were entirely distinct from one another.

In the early Tertiary times the continuity of the northern continent became broken in Central Asia, while the American extension became shorter, and the south-east Asiatic extension became accentuated, taking in what is now the Malay Peninsula and Sumatra. Later, at about the Miocene period, this break in Asia disappeared again, and the northern continent became once more continuous, although a narrow gulf divided North America and Greenland up to the latitude of 75° North. At this time the American southern extension was again shorter, and a new extension in Asia included what is now the Peninsula of India. Meanwhile the southern continent had disintegrated into several masses, only separated by very narrow straits from the northern continent. In the Pliocene further changes occurred. The northern continent was completely broken by a narrow sea between Ellesmere Island and Greenland, but the greatest changes were towards the south. North and South America became joined, as did Africa to Central Asia and India. The Malayan extension increased to include the present Philippines and Java. This configuration remained until the Pleistocene time, since when the present land outlines gradually came into being.

From the point of view of the Magnolieae the following are the chief

points of interest in the foregoing account. First, except for the temporary Eocene break in Central Asia and the narrow gulf between Ellesmere Island and Greenland, initiated in the Pliocene, there has been complete lateral continuity in the northern hemisphere since the Upper Cretaceous. Second, there was a land bridge between Greenland and Europe, via Iceland, till a period late in the Pleistocene. Thirdly, until the Pliocene, there was no connexion between the northern and southern continental masses. Lastly, ever since the Upper Cretaceous, there has been a sea where now the Mediterranean lies. Until the Pleistocene, this marine area was much increased by a considerable Central Asiatic enlargement of the present Black Sea.

The history of one particular area, Central America and the West Indian archipelago, is unsatisfactory. It appears to have undergone greater recent changes than any other part of the world. Opinions differ as to the order and nature of events, but as an example of what may have occurred Harshberger (29) may be quoted. This writer suggests that during the Cretaceous, Central America, the West Indies, and North-South America were all parts of one continent, which broke up at the end of that period. This was chiefly due to the formation of the Caribbean Sea. The northern portion remained a unit during the Eocene. At the end of the Eocene, and during the Oligocene and Miocene, the connexion between the Greater Antilles and North America was broken. This connexion was re-established at the end of the Tertiary, when the Antilles were much larger than they are now and continuous.

Another writer, Spencer (30), more or less reiterates this view. Speaking of the West Indies, he says: ' . . . that of early Pleistocene days, when the elevation reached its maximum height, and all the islands were united so as to connect South and North America.'

These quotations have been made in order to indicate that there is evidence in favour of the view that there was a land connexion between North and South America, via the West Indies, in the late Pliocene or early Pleistocene times. From the point of view of the Magnolieae this is a matter of some importance.

THE GLACIAL EPOCHS.

The Pleistocene glacial epoch was by no means the first or only glacial epoch, but as far as is known there was no extensive glaciation between the later stages of the Palaeozoic era and the Pleistocene. Hence, as far as the Magnolieae are concerned, it is almost certain that if glaciation played a part in their distribution, it did not do so until the Pleistocene.

W. B. Wright (31) has collected together a vast amount of information concerning the Pleistocene glaciation, and this has been the source of much of the following matter.

The glacial epoch is generally considered to have attained its maximum severity comparatively slowly. This is important, since a migrationary hypothesis assumes that the plants concerned were able to keep pace with the advancing ice. A second important point is that, according to most authorities, the glaciation exhibited a more or less marked pulsation, and was divided into several oscillations, which much intensified its effect. Although this is a very widely held view, there is little direct evidence to support it. Recent work by Madsen, Nordmann, and Hartz (32) on the *Cyprina* clays of north-west Europe seems to afford definite proof of at least one such oscillation. Similarly, the evidence shows several distinct glaciations in America. It is also difficult to say whether the ice ever covered areas which it had once left, or whether the orderly retreat was simply broken by a series of prolonged halts. At any rate it seems probable that some kind of oscillation occurred.

Of special interest is the distribution and maximum extent of the ice. Areas affording evidence of past glaciation fall into two categories—those within the main circumpolar ice-cap and those south of it, but which were covered with ice owing to their altitude. These latter are, of course, the various isolated mountain ranges. As the ice-cap itself extended southwards the ice on these mountains extended downwards until, in some cases, considerable areas must have been covered. Due allowance must be made for this, since in the following paragraphs only the main ice-cap is described.

The ice of the Pleistocene attained its maximum extent over North America, where its most southern limit was about 38° North, in the eastern part of the continent. Above this line the whole of the continent was covered, although on the western side the southern limit was some fewer degrees farther north. Some authorities consider that during this maximum glaciation the southern limit moved from east to west. In any case, the presence of the Rocky Mountains would cause a local southern extension, and we may conclude that at this time the whole of North America above 40° North was within the ice-cap.

Passing east, Greenland was completely covered, and the ice extended over the land bridge, by way of Iceland, all over the British Isles, except that part of England south of the River Thames. At about this level the southern limit ran eastwards to the Carpathians, but from this point turned almost due north, and the ice does not seem to have passed a line from the Carpathians to the Timan Mountains in Russia. In North Asia there is very little evidence of glaciation between the Timan Mountains and the extreme north-east corner of Siberia, which latter formed the western edge of the American portion of the ice sheet. In this region two glacial periods appear to have been separated by a warmer time.

If now the extent of the ice during the glacial epoch is plotted upon

a map of the world a feature of great interest is seen, i. e. that the ice did not extend southwards equally in all directions from the Pole. Over Asia the extent was little greater than it is at present. The vast bulk of the ice spread south over Europe and North America, and the geometrical centre of the ice-cap was somewhere in the neighbourhood of Central Greenland and at a latitude of about 75° North.

Mention must here be made of a view discussed by Simmons (33). This is that the ice-cap was not absolutely continuous within its outer limits, but that certain points far north remained ice free, and provided refuges in which relics of the pre-glacial northern flora managed to persist until the retreat of the ice. This idea is interesting, but it is difficult to imagine an area surrounded by vast fields of ice and yet sufficiently temperate to support even a scanty and markedly arctic flora. Even if this were the case it can scarcely have had any effect on such plants as the Magnolieae.

It must also be remembered that although the ice-cap is considered as one simultaneous entity there is great difficulty in correlating the different parts of it. There is no absolute proof that the ice was contemporaneous in America and in Europe, or even in Britain and Europe, but in absence of proof to the contrary it may be assumed that in all directions the southern-most limit of the ice was reached at approximately the same time.

PAST CLIMATIC CONDITIONS.

The general views held as to the past climates of the northern hemi-sphere may be very shortly stated. It is believed that from the Upper Cretaceous until nearly the end of the Tertiary the climate over the whole northern hemisphere was more or less constant and temperate, to some extent paralleling that of the southern United States to-day. According to Eckardt (34) the arctic Tertiary conditions up to 70–80° North were similar to those of Italy to-day. In general it may be concluded that, at that time, the climate of any particular spot in the northern hemisphere was similar to that found to-day 2c–30° farther south. The climate of Southern and Central Europe in the Eocene was like the present climate of Mexico and South-eastern Asia, with such plants as cycads, palms, screw-pines, bamboos, and bananas. At higher levels a more temperate flora prevailed. At this point it may be mentioned that there are indications that the climate immediately after the retreat of the ice was rather warmer than it is at present (post-glacial optimum), and that various plants occupied higher latitudes than they do at present.

As Wright (31) expresses it, it is an hypothesis widely held at the present time that the ice age was an interruption of the normal sequence of climatic evolution. Brooks (35) states that the Tertiary throughout most of its length appears to have been characterized by remarkably mild and

equable climates and conditions, extending into comparatively high latitudes. Huntingdon and Visher (36) express the view that during the greater part of geologic history the earth's climate seems to have been relatively monotonous, and that it is only since the late Tertiary that diversity and complexity have commenced. In short, we may look upon the glacial epoch as providing the only fundamentally important change of climate since the evolution of the flowering plants.

Another aspect of the subject is of importance. This is the comparatively recent date, in geological time, of the glacial epoch. Estimates of geologic time are admittedly unsatisfactory, but in order to emphasize the point at issue recourse may be had to two such estimates. The figure 10,000,000 years has often been quoted as giving some approximation of the time which has elapsed since the Eocene, and similarly 50,000 years is supposed to indicate the time at which the ice was near its maximum. We may well agree with Gilbert (37) when he says, ' When the work of the geologist is finished, and his final comprehensive report written, the longest and most important chapter will be upon the latest and shortest of the geologic periods '. We are but now witnessing the struggle of the plant world to recover from the devastating effects of the glacial epoch.

Even more interesting than the possible date of the oncoming of the ice are the various estimates of the time which has elapsed since the ice retreated. G. F. Wright (38), Dawson (39), Burkitt (40), and Sollas (41) [1] all give estimates, and the evidences on which they are based. All these estimates put the time which has passed at a much lower figure than is generally imagined. Perhaps the calculations of de Geer (42), based upon the structure of certain laminated clays in Sweden, are most convincing. According to this writer the ice retreated, at any rate from South Sweden, not more than 9,000 years ago. Other estimates are of much the same magnitude. As Wright (31) says, it is quite conceivable that while the civilization of Babylon was at its height, those parts of Europe and North America which are now the centres of civilization were covered by a thick mantle of ice.

The important point which emerges from these estimates is that while the ice age did not commence until comparatively late in geologic time, the direct effect of the ice lasted for a period very much longer than the time which has elapsed since its retreat. It is important to realize these various time values, in order to understand the different stages in the life-history of the Magnolias. For example, if the supposition that the North American species followed the retreating ice is correct, it follows that their present distribution in North America has been very recent, and may well even now not have reached completion.

[1] The recently published third edition of Sollar (London, 1924) confirms the former editions and gives a very up-to-date summary of our knowledge of this subject.

CONCLUSIONS.

The various foregoing data may now be correlated, and a life-story of the Magnolieae suggested in the following account.

The Magnolieae were evolved at some period antecedent to the Cretaceous, by which time they had attained a wide distribution over the northern hemisphere, up to and including very high latitudes. From this time, throughout much of the Tertiary period, this wide distribution was maintained—probably without very much contraction. At the end of the Pliocene a great climatic change took place, consequent upon a general lowering of temperature and a spread of ice in the northern hemisphere. This change, which was the first great climatic change in the history of the Magnolieae, or indeed of the flowering plants as a whole, came too rapidly to allow any progressive adaptational changes on the part of the plants, and they were forced to migrate. Since the change of conditions spread from the north the direction of their movement was towards the south. Where a path was open to them they moved on, but where their progress was prevented by climatic or topographic obstacles they were deflected or exterminated. With the subsequent shrinkage of the ice their southward migration was reversed, and in many cases their steps were retraced. It is most probable that the ice advanced and retired more than once, and that the movements of the Magnolieae were similarly repeated. The combined result of all these movements was to leave the plants, on the final retreat of the ice, in the areas where they are now found—these areas being similar in climatic respects to the vastly greater areas which these plants once occupied.

Although applied here to one particular group of plants this hypothesis has no merits of novelty. Reference may here be made to three authors who discuss the matter. Darwin (5) in the ' Origin ' deals with it at length, and considers it most important. Hooker and Thomson (7) comment upon Forbes's essay in the following words : 'We consider the principles embodied to be sound, of universal application, and as necessary to be understood by the student of nature as are the laws of climate and the distribution of heat and cold.' A. R. Wallace (43) also discusses it, but more from the point of view of the distribution of animals.

When the migrationary hypothesis is applied to the individual case of the Magnolieae there are several rather anomalous facts to be explained. It has already been stated that the Magnolieae are not found in certain parts of the northern hemisphere where climatic conditions would seem to be favourable, as, for example, on the Pacific coast of North America. This appears to be due to the fact that, having once migrated from such regions, their return to them is prevented by the nature of the interposing land areas.

On the other hand, the particular climatic of such areas may only have arisen since the departure of the plants from similar latitudes, and the region under discussion may never have supported species of *Magnolia*.

The occurrence of two species in Southern Brazil is remarkable. If the ice ages of the two hemispheres were consecutive and not simultaneous, these plants may be the remnants of those which, in their movements south, passed far down into the southern hemisphere. If, on the other hand, the ice ages of the two hemispheres were contemporaneous, these records may be the result of a subsequent spread south instead of north of species which had accumulated in the tropics during the maximum extent of the ice.

It will have been noticed that the North American Magnolias differ slightly in distribution and climatic relation from the rest of the group. This may be correlated with the fact that this region is the one in which glacial conditions were most severe, where the maximum amount of direct north and south migration was possible without extermination. Some of these species are the only ones which at present occupy areas which, during the Pleistocene, were within the ice-cap.

Returning to Brazil, while the hypothesis may explain the presence of species in Southern Brazil, it does not explain their absence in Northern Brazil. It must be remembered that the present absence of species does not mean that they were always absent, or that they never reached such places. In this case there is entire absence of palaeontological evidence, even of a negative kind. Further, many of the Magnolieae are very rare and little known, a remark which may also be applied to the flora of Brazil as a whole. It is most reasonable to suppose that the apparent absence of Magnolieae is due to excessive competition with other indigenous plants or to our lack of knowledge of the region. There may well be isolated species maintaining a precarious hold in places hitherto untouched by collectors.

The whole migrationary hypothesis is based upon the assumption that plants in general are unable to acclimatize themselves to rapid changes of climate, and that their optimal environmental conditions are comparatively rigid. The acceptance of this assumption has very far-reaching effects. It infers, first, that the present climatic relations of plants are those to which they have always been accustomed. Using the Magnolieae as an example, it may be assumed that the climate of most of the northern hemisphere during the later Cretaceous and at least the beginning of the Tertiary was fairly constant, and similar to that of those regions of the modern world in which the Magnolieae are found to-day. This reasoning pushed farther opens wide possibilities, not only as regards the elucidation of past climates, but also as regards the place of origin of various plant types.

Another point in which the hypothesis is of value is in such a problem as that of the similarity between the floras of south-east North America and

South-east Asia, discussed many years ago by Asa Gray (13) and Hooker. The genus *Magnolia* is an example of this, and can be explained on the grounds that these plants are the natural remnants of a once widespread or circumpolar genus. But many other plants have a very similar story, and this explanation is applicable to them as well as to the Magnolias.

The hypothesis also explains the very large numbers of Magnolieae in the Indo-Chinese region. This region was little affected by the glacial epoch, and presumably contains not only its own original endemic species, but also a certain number which reached it by lateral migration from other parts of Asia.

Finally, the geological and climatic evidence points to the fact that arctic conditions, such as we know now, are of very recent geological advent. If this is so, then the arctic floras must be some of the most recent of the world's floral elements. Yet arctic plants are among the most widely distributed.

In the foregoing paragraphs the migrationary hypothesis has been applied to a definite group of plants, and has been shown to be capable of explaining all the known data with the minimum of supposition. Despite its age, the subsequent increase of our knowledge and the reversal of old conceptions have served merely to increase its value. It now remains to consider the position of the hypothesis of Age and Area. The two hypotheses are incompatible when applied to similar cases—if one is true the other must be false. It must be said at once that Willis, in his postulation, insists on several reservations. One of these particularly bears upon the migrationary hypothesis; he points out that Age and Area only holds so long as conditions remain reasonably constant, and may be enormously modified by changes of climate from one region to the next. The truths of the main facts concerning the glacial epoch can hardly be challenged at the present time, and hence Age and Area cannot be applied in cases where glacial migration has had an influence upon plant distribution. The effect of this reservation is, then, that Age and Area cannot be applied to any considerable portion of the present flora of the northern hemisphere. The only plants of this area with which it can possibly deal are those which may have arisen in the extremely short time which has elapsed since the passing of the glacial epoch, or which were, by a rare combination of circumstances, unaffected by that revolution.

The position may be summed up finally in the following sentences: Forbes's hypothesis of Climatic Migrations, since it takes into account the effects of the various glacial oscillations upon plant distribution, has a very wide and general application, and is of the utmost value in the solution of many distributional problems. In the comparatively few cases, however, in which glacial or other climatic effects have not been felt it cannot apply, and here its position may possibly be taken by Dr. Willis's hypothesis of Age

and Area. The two hypotheses together are capable of explaining very many, if not all, of the problems of distribution in plants, but while that of Migrations is of very wide application, that of Age and Area only serves to explain certain rather abnormal and comparatively rare cases.

SUMMARY.

1. The Magnolieae are considered in this paper as being composed of the three genera, *Magnolia*, *Talauma*, and *Michelia*. *Liriodendron* is not discussed.

2. The present distribution of these plants is partly continental and partly insular in the south-east corners of the two great land masses of the northern hemisphere.

3. Their present distribution is correlated with a particular combination of climatic conditions.

4. Their past distribution during the Cretaceous and early Tertiary extended almost all over the northern hemisphere.

5. This wide distribution was maintained until the Pleistocene, when they were forced to migrate in face of the climatic changes.

6. The Pleistocene ice retreated, geologically speaking, only a very short time ago, and the return movements of the plants are probably not yet complete.

7. Our present knowledge of palaeogeography accords with the possibility of such movements.

8. The present distribution of the Magnolieae is the direct result of the enforced migration due to the great climatic changes in the Pleistocene.

9. Many of the plants of the northern hemisphere have a history similar to that of the Magnolieae, and hence the hypothesis of Age and Area can have but a very limited application when applied to this region.

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The Problem of Sex in Coprinus lagopus.

BY

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With fifteen Tables in the Text.

I. INTRODUCTION.

THE problem of sex in the Basidiomycetes has occupied the atten-
tion of mycologists for more than one hundred and fifty years, and
at various times during that period has proved to be a most fruitful
subject for speculation and research. The earlier mycologists confined
their efforts almost wholly to a search for sexual organs analogous to
those present in the Flowering Plants. After much careful study, however,
the early theories of sexuality have been abandoned, and the conclusion is
now generally accepted that in the Basidiomycetes the last trace of morpho-
logical sexual differentiation has disappeared.

With recent advances in cytology, attention has been directed towards
the nuclear phenomena which occur during the life-histories of many of the
higher fungi. In 1892, Rosen proved that in the aecidiospores and uredo-
spores of *Uromyces pisi* and *Puccinia anserina* there are present two nuclei,
while in the mature teleutospores there is present but a single nucleus. He
also studied the gill-cells of several species of Autobasidiomycetes, and
found that in the young gills of *Armillaria mucida* the nuclei are
numerous and disposed in pairs, but that as the gills become more mature,
the nuclei increase in size and decrease in number, until finally, each
basidium comes to contain but a single nucleus. Rosen's observations
were later confirmed and extended by the work of Dangeard, Sappin-
Trouffy, Maire, Blackman, and others, with the result that there has been
built up a new and promising theory of sexuality in the Basidiomycetes
based entirely on nuclear phenomena.

In the Hymenomycetes, that great group of the Basidiomycetes which
includes the gilled fungi, it has been shown that, prior to the production of
the fruit-body, individual cells of the vegetative mycelium come to possess
one or more pairs of nuclei. As the mycelium develops, the pairs of
nuclei undergo a series of conjugate divisions, until finally, just before the

production of the basidium, the number of nuclei in each cell is reduced to a single pair which unite to form the fusion nucleus of the basidium. The fusion of the two nuclei in the basidium has been regarded by Dangeard and others as a reduced sexual act equivalent, nevertheless, to the fertilization which takes place in the higher plants.

The origin of the paired nuclei which first appear in the vegetative mycelium was still to be demonstrated. In 1918 light was thrown on this problem by the researches of Mlle Bensaude, entitled ' Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes'. Mlle Bensaude (1) showed that *Coprinus fimetarius*, one of the Hymenomycetes, resembles certain Mucorini investigated by Blakeslee in that it is *heterothallic*. Two cultures of *Coprinus fimetarius* were obtained, each of which had originated from a single spore. When sub-cultures from these mycelia were grown separately, they remained for eight months in the *primary* condition, i.e. the mycelium branched irregularly, produced no clamp-connexions, and possessed nuclei disposed singly and not in pairs ; furthermore, the mycelia were completely sterile, producing no fruit-bodies whatever. When the two mycelia were brought together and allowed to fuse, they soon developed a *secondary* mycelium characterized by regular branching, the presence of clamp-connexions, the paired condition of the nuclei, and the perfect production of fruit-bodies. Mlle Bensaude (1) was able to show that the paired nuclei of the secondary mycelium divide conjugately, and that each division of the dicaryon is associated with the formation of a septum with a clamp-connexion. Thus, it appeared that the two nuclei which finally fuse in each basidium of *Coprinus fimetarius* are the direct descendants of a single pair of nuclei derived from two different mycelia of opposite sex.

Further research by Kniep, Miss Mounce, and Vandendries has greatly extended the work of Mlle Bensaude. Kniep (5) has shown that *Schizophyllum commune* is a heterothallic species, but that in this species fruit-bodies are sometimes produced from monosporous mycelia. Thus, the phenomenon of heterothallism is not necessarily associated with the sterility of monosporous mycelia. Kniep found, however, that the spores originating from such monosporous fruit-bodies were all of the same sex, and that when sown in polysporous culture the mycelium to which they gave rise never produced clamp-connexions. Therefore, while in a heterothallic species fruit-bodies may develop from monosporous mycelia, such fruit-bodies produce spores which are all of one sex.

Both Kniep (4) and Mlle Bensaude (1) have found that the presence of clamp-connexions is invariably associated with the conjugate division of the nuclei. This fact furnishes a reliable criterion for determining whether a given species of Hymenomycete is homothallic or heterothallic. If clamp-connexions appear regularly on individual mycelia of mono-

sporous origin, the species must be homothallic; while if no clamp-connexions appear on such monosporous mycelia, but only on compound mycelia resulting from the union of two mycelia of opposite sex, the species must be heterothallic.

Miss Mounce (9, 10) has shown that *Coprinus sterquilinus* and *Coprinus stercorarius* are homothallic, and that *Coprinus lagopus* and *Coprinus nivens* are heterothallic. Thus, in a single genus, there exist side by side homothallic and heterothallic species.

The following study was undertaken with the object of further extending our knowledge of the sex of *Coprinus lagopus*. For several reasons this species is particularly well suited to an investigation of this kind. It is a well-known species, and occurs regularly on horse-dung cultures both in North America and in Europe. In the laboratory at Winnipeg, *Coprinus lagopus* appeared on seven out of eight cultures of dung obtained from widely separated points in Canada and England. The culture of dung which failed to produce fruit-bodies of *Coprinus lagopus* had been exposed to the weather for a long time, and produced little fungous growth of any kind. The spores are black, and, therefore, easily seen, and a high percentage of them germinate. Finally, the species requires only from twelve to fourteen days to complete its life-cycle from spore to spore, a circumstance which is of great importance wherever it is necessary to continue the study of sex throughout several successive generations.

II. Methods.

Miss Mounce and Kniep have both studied the sex of *Coprinus stercorarius*, but whereas Miss Mounce (9, 10) found this species to be homothallic, Kniep (5) asserts that it is heterothallic. In view of such contradictory findings, and also as a possible explanation of the varying results which she obtained at different times for *Coprinus lagopus*, Miss Mounce (10) suggested that there may exist both homothallic and heterothallic strains of the same species. In order to investigate this possibility, and, at the same time, to obtain results which will be general and not local in application, fruit-bodies of *Coprinus lagopus* used in the present study have been secured from a number of places widely separated from one another. With the object of securing fruit-bodies, samples of dry horse-dung were obtained from the following places in Canada: (1) Vancouver, British Columbia; (2) Edmonton, Alberta; (3) Shellbrook, Saskatchewan; (4) Winnipeg, Manitoba; and (5) Halifax, Nova Scotia. At a later date, samples were obtained by Professor A. H. R. Buller from three different places at Birmingham, England. Thus, in all, eight different samples of dung were collected at the laboratory in Winnipeg. Each lot of dung

was placed in a crystallizing dish, moistened with tap-water, covered with a closely fitting glass plate, and set upon the laboratory table. In from ten to fourteen days, fruit-bodies of *Coprinus lagopus* made their appearance on all the different lots of dung except the one from Halifax, Nova Scotia. The failure of this culture to produce fruit-bodies of *Coprinus lagopus* was no doubt due to its extremely exhausted condition. The fruit-bodies which came up on the other seven cultures answered to the description of *Coprinus lagopus* given by Lange (8) in his monograph on the genus *Coprinus*, and also resembled in appearance the illustrations of *Coprinus lagopus* drawn by Brefeld (2). If further information regarding this species is required, reference should be made to Buller's 'Researches on Fungi', vol. iii, 1924, where a detailed study of *Coprinus lagopus* will be found, together with numerous photographs and drawings.

Spore-deposits from one or more fruit-bodies of each culture were collected on dry sterilized glass slides, care being taken that spores from only a single fruit-body fell on each slide. The slides were then labelled and kept in a large covered cardboard case so constructed that the slides were well separated from one another. These spore-deposits, originating from different points in England and Canada, provided very suitable material for the study of sex in *Coprinus lagopus*.

All monosporous cultures were made by means of the dry-needle method (3), which has been described in a separate paper. The spores of *Coprinus lagopus* germinate readily in dung-agar, and, while there is some difference in the viability of spores from different fruit-bodies, the average germination throughout the whole of this work was between 80 and 90 per cent. With certain fruit-bodies, it was not uncommon to find that of ten spores isolated and placed in hanging-drops of dung-agar, every one had germinated within twenty-four hours. When the mycelium had grown out into the agar, the drop containing the spore and the mycelium was transferred to a Petri dish into which had been poured a layer of sterile dung-agar about 2 m.m. in thickness. A circular patch of mycelium grew out from the drop of agar and increased rapidly in size until, at the end of four days, it was generally large enough to be used for pairing with other mycelia. If the monosporous mycelia obtained in this way were required to be kept for some time, transfers were made from the Petri dishes to glass tubes 3 inches long and $\frac{9}{10}$ of an inch in diameter, which had previously been about half filled with fresh horse-dung, plugged with cotton-wool, and sterilized in an autoclave for one hour at fifteen pounds pressure.

When the pairings were to be made, a small piece of the dung-agar about 1 c.m. square, bearing some of the monosporous mycelium, was transferred by means of a sterile platinum loop to a Petri dish containing a layer of sterile dung-agar. Another piece of agar was similarly transferred from

a second monosporous mycelium and placed beside the first. At the end of six days, the circular patch of mycelium which had grown outward from the pieces of agar was examined for clamp-connexions. A brief macroscopic examination of the mycelium as a whole was generally sufficient to determine whether or not a secondary mycelium bearing clamp-connexions had developed, but in all cases a final microscopic examination of the cultures was made. While most of the pairings of monosporous mycelia were made in the manner just described, some of those recorded in Table VII were made in tubes of sterile horse-dung. Since the mycelia used in these pairings had been previously transferred to tubes of sterile horse-dung, the operation of pairing was easily effected by removing from the culture tubes small pieces of the dung containing mycelium and placing them together in a third tube of sterile horse-dung. At the end of six days some of the compound mycelium was taken from the tube, mounted in water on a glass slide, and examined under a microscope for clamp-connexions. As the mycelium of *Coprinus lagopus* grows equally well on either dung-agar or sterile dung, the results obtained by the two methods of pairing are perfectly comparable, but the ease with which pairings can be made in Petri dishes gives the former method a distinct advantage.

The dung-agar used for spore germination and for the growing of different mycelia which were about to be paired was made in the following manner : A quantity of fresh horse-dung was placed in a large enamel dish and tap-water was added at the rate of 1,000 c.c. to 300 grm. of dung. The mixture was well stirred, and the resulting liquid was decanted off and strained through cheesecloth to remove the larger particles which it contained. This liquid was then boiled and filtered through cotton-wool. To the decoction thus obtained, agar at the rate of 1·7 per cent. was added and the mixture was boiled until the agar had melted. After filtering again through cotton-wool, the mixture was poured into a series of test-tubes, which were subsequently plugged with cotton-wool and sterilized in the autoclave for one hour at fifteen pounds pressure. On a few occasions, the dung-agar was neutralized with ammonium hydroxide, but this procedure proved to be unnecessary as the mycelium of *Coprinus lagopus* seemed to grow equally well on dung-agar which had not been neutralized.

III. EXPERIMENTAL.

(1) *All possible Pairings of Monosporous Mycelia from Individual Wild Fruit-bodies.*

By pairing together a number of monosporous mycelia of *Coprinus lagopus*, Miss Mounce (10) found that while some unions gave rise to clamp-connexions, others produced no clamp-connexions whatever, but, unlike certain of the Mucorini investigated by Blakeslee, there appeared to be no strict segregation into (+) and (−) strains. In the present study, mono-

sporous mycelia have been obtained from the spores of several wild fruit-bodies which came up on the dung cultures collected from different places.

		AB	AB	AB	ab	ab	ab	ab	Ab	Ab	aB
		51	52	54	55	57	58	59	50	56	53
AB	51	—	—	—	+	+	+	+	—	—	—
	52	—	—	—	+	+	+	+	—	—	—
	54	—	—	—	+	+	+	+	—	—	—
ab	55	+	+	+	—	—	—	—	—	—	—
	57	+	+	+	—	—	—	—	—	—	—
	58	+	+	+	—	—	—	—	—	—	—
	59	+	+	+	—	—	—	—	—	—	—
Ab	50	—	—	—	—	—	—	—	—	—	+
	56	—	—	—	—	—	—	—	—	—	+
aB	53	—	—	—	—	—	—	—	+	+	—

TABLE I.   *Coprinus lagopus.*   All possible pairings of ten monosporous mycelia from fruit-body No. 1 (Vancouver).

		A^2B^2	A^2B^2	A^2B^2	A^2B^2	a^2b^2	a^2b^2	a^2b^2	a^2B^2	a^2B^2	a^2B^2	A^2b^2
		25	26	27	28	20	23	24	21	29	30	16
A^2B^2	25	—	—	—	—	+	+	+	—	—	—	—
	26	—	—	—	—	+	+	+	—	—	—	—
	27	—	—	—	—	+	+	+	—	—	—	—
	28	—	—	—	—	+	+	+	—	—	—	—
a^2b^2	20	+	+	+	+	—	—	—	—	—	—	—
	23	+	+	+	+	—	—	—	—	—	—	—
	24	+	+	+	+	—	—	—	—	—	—	—
a^2B^2	21	—	—	—	—	—	—	—	—	—	—	+
	29	—	—	—	—	—	—	—	—	—	—	+
	30	—	—	—	—	—	—	—	—	—	—	+
A^2b^2	16	—	—	—	—	—	—	—	+	+	+	—

TABLE II.   *Coprinus lagopus.*   All possible pairings of eleven monosporous mycelia from fruit-body No. 2 (Edmonton).

The results of pairing together monosporous mycelia derived from individual fruit-bodies are given in Tables I–VI. Fruit-body No. 1 (Table I) came from Vancouver; fruit-body No. 2 (Table II) from Edmonton; fruit-body

No. 3 (Table III) from Shellbrook; and fruit-bodies Nos. 4, 5, and 6 (Tables IV, V, and VI) from Winnipeg. Fruit-bodies Nos. 5 and 6 were

		A^3B^3			a^3b^3			a^3B^3			A^3b^3
		41	43	44	35	42	47	36	37	39	46
A^3B^3	41	—	—	—	+	+	+	—	—	—	—
	43	—	—	—	+	+	+	—	—	—	—
	44	—	—	—	+	+	+	—	—	—	—
a^3b^3	35	+	+	+	—	—	—	—	—	—	—
	42	+	+	+	—	—	—	—	—	—	—
	47	+	+	+	—	—	—	—	—	—	—
a^3B^3	36	—	—	—	—	—	—	—	—	—	+
	37	—	—	—	—	—	—	—	—	—	+
	39	—	—	—	—	—	—	—	—	—	+
A^3b^3	46	—	—	—	—	—	—	+	+	+	—

TABLE III. *Coprinus lagopus.* All possible pairings of ten monosporous mycelia from fruit-body No. 3 (Shellbrook).

		A^4B^4			a^4b^4	A^4b^4				a^4B^4				
		4	7	8	5	2	6	10	11	1	3	9	M1	M2
A^4B^4	4	—	—	—	+	—	—	—	—	—	—	—	—	—
	7	—	—	—	+	—	—	—	—	—	—	—	—	—
	8	—	—	—	+	—	—	—	—	—	—	—	—	—
a^4b^4	5	+	+	+	—	—	—	—	—	—	—	—	—	—
A^4b^4	2	—	—	—	—	—	—	—	—	+	+	+	+	+
	6	—	—	—	—	—	—	—	—	+	+	+	+	+
	10	—	—	—	—	—	—	—	—	+	+	+	+	+
	11	—	—	—	—	—	—	—	—	+	+	+	+	+
a^4B^4	1	—	—	—	—	+	+	+	+	—	—	—	—	—
	3	—	—	—	—	+	+	+	+	—	—	—	—	—
	9	—	—	—	—	+	+	+	+	—	—	—	—	—
	M1	—	—	—	—	+	+	+	+	—	—	—	—	—
	M2	—	—	—	—	+	+	+	+	—	—	—	—	—

TABLE IV. *Coprinus lagopus.* All possible pairings of eleven monosporous mycelia from fruit-body No. 4 (Winnipeg), and two polysporous mycelia, *M* 1 and *M* 2, from a monosporous fruit-body of mycelium No. 1.

collected at the same time and from the same dish of dung, while fruit-body No. 4 was obtained from a second dish of dung brought from the same stable. The sign (+) in all of the tables indicates that clamp-connexions appeared after the union of the two mycelia ; the sign (−) indicates that no clamp-connexions appeared after the union.

		A^5B^5			a^5b^5				A^5b^5		a^5B^5	
		60	62	67	61	64	66	68	63	65	69	70
A^5B^5	60	—	—	—	+	+	+	+	—	—	—	—
	62	—	—	—	+	+	+	+	—	—	—	—
	67	—	—	—	+	+	+	+	—	—	—	—
a^5b^5	61	+	+	+	—	—	—	—	—	—	—	—
	64	+	+	+	—	—	—	—	—	—	—	—
	66	+	+	+	—	—	—	—	—	—	—	—
	68	+	+	+	—	—	—	—	—	—	—	—
A^5b^5	63	—	—	—	—	—	—	—	—	—	+	+
	65	—	—	—	—	—	—	—	—	—	+	+
a^5B^5	69	—	—	—	—	—	—	—	+	+	—	—
	70	—	—	—	—	—	—	—	+	+	—	—

TABLE V. *Coprinus lagopus.* All possible pairings of eleven monosporous mycelia from fruit-body No. 5 (Winnipeg).

		A^6B^6		a^6b^6		A^6b^6	a^6B^6
		73	76	74	75	71	72
A^6B^6	73	—	—	+	+	—	—
	76	—	—	+	+	—	—
a^6b^6	74	+	+	—	—	—	—
	75	+	+	—	—	—	—
A^6b^6	71	—	—	—	—	—	+
a^6B^6	72	—	—	—	—	+	—

TABLE VI. *Coprinus lagopus.* All possible pairings of six monosporous mycelia from fruit-body No. 6 (Winnipeg).

In each of the above tables, it will be seen that the mycelia fall into our distinct groups. The tables have been rearranged by collecting together the mycelia belonging to each group ; for example, in Table V, mycelia 60, 62, and 67 belong to a first group, 61, 64, 66, and 68 to a second, 63 and 65 to a third, and 69 and 70 to a fourth. Furthermore, it will be seen that the mycelia of the first group react with those of the second, and those of the

third react with those of the fourth, but the mycelia of the first and second groups fail to react with those of the third and fourth groups. These results show that the spores of a single fruit-body of *Coprinus lagopus*, while alike morphologically, may be divided sexually into four distinct groups regardless of where the fruit-body producing the spores is obtained. Miss Mounce's suggestion that there may exist both homothallic and heterothallic strains of *Coprinus lagopus* has not been supported by this study, as all strains of *Coprinus lagopus* investigated have proved to be heterothallic.

Kniep (7) obtained tables for *Schizophyllum commune* similar to those above, and explained his results on the theory that sex is determined in this species by two pairs of Mendelian factors (*A a*) and (*B b*). The same theory will explain the sexual reactions of *Coprinus lagopus*. We may suppose that in this fungus, when two haploid mycelia of opposite sex unite and form a diploid mycelium, of each pair of nuclei present in each cell, one has been derived from one haploid mycelium and the other from the other haploid mycelium, i. e. they are of opposite sex. Finally, as a result of conjugate nuclear divisions of the first-formed nuclear pairs, each basidium comes to contain a pair of nuclei of opposite sex. In each basidium the nuclei fuse together, and then the fusion nucleus divides twice with chromosome reduction, with the result that the four nuclei produced are haploid, each of them containing one of the factors (*A a*) and one of the factors (*B b*). The four nuclei pass into the four spores so that, in the end, the spores come to be of the same sex as the nuclei which enter them. From the sexual point of view, therefore, it is possible to have from a single fruit-body but four kinds of spores : (*A B*), (*a b*), (*A B*), and (*a B*).

The mycelia arising from these spores will fall, therefore, into four groups. Those with the genetic constitution (*A B*) will unite with those designated as (*a b*). but with no others, as a union with (*A b*) or (*a B*) would bring together two like factors ; for a similar reason (*A b*) will unite only with (*a B*).

In accordance with the above theory, it has been possible to assign sex factors to each of the groups in Tables I to VI. An examination of these tables will show that, wherever two mycelia possess a common factor, no union has taken place. but where no common factor is present, a union of the two mycelia has occurred resulting in the formation of clamp-connexions.

(2) *The Pairing of Monosporous Mycelia from Different Wild Fruit-bodies.*

Since each of the six fruit-bodies studied was found to possess spores giving rise to four different kinds of mycelia, the following question may now be raised : Are the sexual groups in all of these fruit-bodies identical, or are they different ? For example, are mycelia 25, 26, 27, and 28 of Table II the same sexually as mycelia 4, 7, and 8 of Table IV ? In order to answer this question, eleven mycelia of fruit-body No. 2 (Table II) were

paired with eleven mycelia of fruit-body No. 4 (Table IV). The results of the pairings, given in Table VII, show that there is *complete fertility* between monosporous mycelia derived from these two wild fruit-bodies. In order to verify this result, a large number of pairings were made between monosporous mycelia of different fruit-bodies. Space does not permit of all these results being presented in tabular form, but a list of the pairings made is given below :

100	pairings between fruit-body			No. 1	and fruit-body	No. 3
100	,,	,,	.,	No. 1	,,	No. 2
100	.,	,,	,,	No. 1	,,	No. 4
100	,,	,,	,,	No. 4	,,	No. 3
100	,,	,,	.,	No. 2	,,	No. 3
36	,.	,,	,,	No. 5	,,	No. 6
12	,.	.,	,,	No. 5	,,	No. 4
12	,,	,,	.,	No. 6	,.	No. 4
8	.,	,,	,.	No. 5	,.	No. 1
8	,,	,,	,,	No. 6	,,	No. 1
14	,,	,,	,,	No. 5	.,	No. 2
14	,,	,,	,,	No. 6	.,	No. 2
10	,,	,,	,,	No. 5	,,	No. 3
10	,,	.,	,,	No. 6	,,	No. 3
8	,,	,,	a Birmingham fruit-body	and fruit-body		No. 1
14	.,	,,	,,	.,	.,	No. 2
10	,,	,,	,,	,,	,,	No. 3
14	.,	,.	,,	,,	,.	No. 4
12	,.	,,	,,	,,	,,	No. 5
12	,,	.,	,,	,,	,,	No. 6

In all of the 694 pairings clamp-connexions appeared regularly, thus showing that the sexual groups in all of these fruit-bodies must be different, and that complete fertility results when monosporous mycelia from different wild fruit-bodies are paired together. In the six fruit-bodies studied in Tables I to VI, there have been established therefore, not four, but twenty-four distinct sexual groups. The complete fertility between fruit-bodies 5 and 6 is especially remarkable, since both were found growing side by side in the same dung culture. While it is possible that the spores giving rise to these two fruit-bodies came from widely different districts, it is nevertheless interesting to know that two and possibly many sexual strains of *Coprinus lagopus* exist side by side within a very small area.

In the list of pairings given above, it is also noteworthy that monosporous mycelia from a fruit-body of *Coprinus lagopus* originating in Birmingham, England, have reacted with monosporous mycelia from fruit-bodies of the same species collected at different places in Canada. The fact

that hyphal fusions took place between the English and Canadian strains, resulting in the formation of clamp-connexions, furnishes a conclusive proof that the *Coprinus lagopus* found in England is identical with the species occurring in Canada. In the appearance of the fruit-bodies and spores, as well as in the habit of growth and general appearance of the mycelium, the English strain of *Coprinus lagopus* was in no respect different from the Canadian species. Five monosporous mycelia of the English strain were kept in culture on dung-agar and in tubes of sterile dung for nearly a month, and while they produced an abundance of the characteristic oidia and gave

		A⁴B⁴			a⁴b⁴		A⁴b⁴			a⁴B⁴		
		4	7	8	5	2	6	10	11	1	3	9
A²B²	25	+	+	+	+	+	+	+	+	+	+	+
	26	+	+	+	+	+	+	+	+	+	+	+
	27	+	+	+	+	+	+	+	+	+	+	+
a²b²	28	+	+	+	+	+	+	+	+	+	+	+
	20	+	+	+	+	+	+	+	+	+	+	+
	23	+	+	+	+	+	+	+	+	+	+	+
	24	+	+	+	+	+	+	+	+	+	+	+
a²B²	21	+	+	+	+	+	+	+	+	+	+	+
	29	+	+	+	+	+	+	+	+	+	+	+
	30	+	+	+	+	+	+	+	+	+	+	+
A²b²	16	+	+	+	+	+	+	+	+	+	+	+

TABLE VII. *Coprinus lagopus.* The pairing of eleven monosporous mycelia from fruit-body No. 2 (Edmonton) with eleven monosporous mycelia from fruit-body No. 4 (Winnipeg).

rise to numerous imperfect fruit-bodies, no clamp-connexions ever appeared. We may, therefore, conclude that the strain of *Coprinus lagopus* found in England is similar to the Canadian species in that it is heterothallic.

Kniep (7) paired monosporous mycelia from different wild fruit-bodies of *Schizophyllum commune* collected at some distance from one another, and found complete fertility between them. Vandendries (12) obtained a similar result with two wild fruit-bodies of *Panaeolus campanulatus*. Further investigations may show that these sexual strains are to be found generally throughout the heterothallic Basidiomycetes, and that each species is made up of a definite number of such strains. On the contrary, the sex-factors for a given species may be undergoing frequent mutations with the result that new sexual strains are continually appearing. Kniep (7) holds the latter view, and has found a considerable amount of evidence to show that

the sex-factors in *Schizophyllum commune* undergo frequent mutations. Thus, for example, a fruit-body possessing the sex-factors *A*, *B*, *a*, and *b* may give rise to some spores having the mutant factors *A′*, *B′*, *a′*, and *b′*, and in this way a new sexual strain would arise with spores belonging to four new groups which would show complete fertility with the old strain possessing the factors *A*, *B*, *a*, and *b*

(3) *The Pairing of Monosporous Mycelia from two Fruit-bodies arising from the same Compound Mycelium.*

(a) *Where the two spores giving rise to the compound mycelium were obtained from the same fruit-body.*

In the preceding experiments, it was found that whenever any two monosporous mycelia from different wild fruit-bodies were brought together, clamp-connexions always appeared. In view of this finding. it becomes of interest to determine whether monosporous mycelia from any two fruit-bodies whatsoever will react in this way.

The simplest case to consider is where the two fruit-bodies have arisen from a compound mycelium produced by the union of two monosporous mycelia of opposite sex which have been obtained from the same fruit-body. If, during the development of the fruit-body and the formation of spores, the various nuclear divisions take place in an orderly manner, it might be expected that, while any number of fruit-bodies might arise from a bisporous mycelium, only four kinds of spores would be formed. If, on the contrary, the nuclear divisions are accompanied by numerous mutations, different kinds of fruit-bodies might arise from the same bisporous mycelium, with the result that the spores from·any two fruit-bodies might not fall into the same sexual groups. Separate spore-deposits were, therefore, obtained from two fruit-bodies, *A* and *B*, which had arisen on a compound ,mycelium produced by the union of mycelia 54 and 58 of Table I.

Four monosporous mycelia were isolated from fruit-body *A*, and ten from fruit-body *B*. The fourteen mycelia were then paired together in all possible ways as shown in Table VIII. In each fruit-body the spores have proved to be of four kinds, but on a closer examination of the table it will be seen that the four groups of fruit-body *A* are identical with those of fruit-body *B*. In other words, the fourteen mycelia have reacted together in every respect as though they had been isolated from a single fruit-body.

(b) *Where the two spores giving rise to the compound mycelium were obtained from different wild fruit-bodies.*

Mycelium 46 of Table III and mycelium 6 of Table IV were paired together, and from this compound mycelium two fruit-bodies, *C* and *D*, were produced. Twenty-six monosporous mycelia were then isolated, thirteen

from fruit-body *C* and thirteen from fruit-body *D*, and all possible pairings were made between them.

It has already been shown that mycelia 46 and 6 belong to different sexual strains which proved to be completely fertile when paired together; they must, therefore, have no sex-factor in common. Since six groups are possible when four different things are combined two at a time, it might be expected that the spores from fruit-bodies *C* and *D* would fall into not four, but six groups.

		A				B									
		AB	ab	Ab	aB	AB				a.b				Ab	aB
		1	2	3	4	2	5	8	9	4	6	7	10	3	1
A	AB 1	—	+	—	—	—	—	—	—	+	+	+	+	—	—
	ab 2	+	—	—	—	+	+	+	+	—	—	—	—	—	—
	Ab 3	—	—	—	+	—	—	—	—	—	—	—	—	—	+
	aB 4	—	—	+	—	—	—	—	—	—	—	—	—	+	—
B	AB 2	—	+	—	—	—	—	—	—	+	+	+	+	—	—
	AB 5	—	+	—	—	—	—	—	—	+	+	+	+	—	—
	AB 8	—	+	—	—	—	—	—	—	+	+	+	+	—	—
	AB 9	—	+	—	—	—	—	—	—	+	+	+	+	—	—
	ab 4	+	—	—	—	+	+	+	+	—	—	—	—	—	—
	ab 6	+	—	—	—	+	+	+	+	—	—	—	—	—	—
	ab 7	+	—	—	—	+	+	+	+	—	—	—	—	—	—
	ab 10	+	—	—	—	+	+	+	+	—	—	—	—	—	—
	Ab 3	—	—	—	+	—	—	—	—	—	—	—	—	—	+
	aB 1	—	—	+	—	—	—	—	—	—	—	—	—	+	—

TABLE VIII. *Coprinus lagopus*. All possible pairings of fourteen monosporous mycelia from fruit-bodies *A* and *B* of mycelium 54 × 58.

From the results of the pairings given in Table IX, it will be seen that, notwithstanding the hybrid nature of fruit-bodies *C* and *D*, only four sexual groups have been found, and that the same groups are present in both fruit-bodies. The two sexual hybrids *C* and *D* are, therefore, similar to the two fruit-bodies *A* and *B* of the last experiment, and bear the same relationship to each other.

If we assume that the nuclei of mycelium 46 carried the sex-factors ($A^3 b^3$), and those of mycelium 6 the sex-factors ($A^4 b^4$), it should follow that the fusion nuclei of the basidia of fruit-bodies *C* and *D* would have the genetic constitution ($A^3 b^3 A^4 b^4$). But, since these fruit-bodies gave rise to only four kinds of spores, we must conclude that, when the reduction

division occurred just before spore formation, no union took place between factors A^3 and A^4 and between factors b^3 and b^4. In other words, although A^3 and A^4 and b^3 and b^4 were sufficiently unlike to permit of the nuclei containing them becoming associated and dividing conjugately with the formation of clamp-connexions, they nevertheless retained a certain likeness

TABLE IX. *Coprinus lagopus.* All possible pairings of twenty-six monosporous mycelia from fruit-bodies *C* and *D* of mycelium 46 × 6

which exerted a repulsive influence and prevented any unions taking place between them during the segregation of sex-factors.

As a result of the experiments just recorded, we may draw the conclusion that when any two monosporous mycelia of *Coprinus lagopus* are brought together so as to form a secondary compound mycelium, fruit-bodies arising from this mycelium will possess spores belonging to but four sexual groups, regardless of the number of fruit-bodies produced. In Table IX an exception must be noted in the case of the union between

mycelia 9 and 3 of fruit-body *C*. These two mycelia reacted very feebly and produced clamp-connexions only on a single hypha, although the culture grew vigorously and was kept under observation for nine days. This deviation from the normal may be regarded as a mutation, similar to those which Kniep (7) found to occur in *Schizophyllum commune*.

(4) *Sexual Relationship between Parent Mycelia and Spores of a First-generation Fruit-body.*

If, during the development of the fruit-body and the formation of spores, the sex-factors segregate out in a regular manner, it might be

		A^2B^2		A^4B^4		A^2B^4		A^4B^2	
		81	85	82	64	80	94	83	87
A^2B^2	81	—	—	+	+	—	—	—	—
	85	—	—	+	+	—	—	—	—
A^4B^4	82	+	+	—	—	—	—	—	—
	84	+	+	—	—	—	—	—	—
A^2B^4	80	—	—	—	—	—	—	+	+
	94	—	—	—	—	—	—	+	+
A^4B^2	83	—	—	—	—	+	+	—	—
	87	—	—	—	—	+	+	—	—

TABLE X. *Coprinus lagopus.* All possible pairings of eight monosporous mycelia from fruit-body *E* of mycelium 25 × 7.

expected that monosporous mycelia from first-generation fruit-bodies would react with the parent mycelia strictly in accordance with the theory of dihybridism. On the contrary, if mutations take place frequently, any abnormalities should appear when the monosporous mycelia from first-generation fruit-bodies are crossed with their parents.

By pairing mycelium 7 of fruit-body 4 (Table IV) with mycelium 25 of fruit-body 2 (Table II), a hybrid fruit-body *E* was obtained. From the spores of this fruit-body eight monosporous mycelia were isolated and all possible pairings were made between them. The results of the pairings are given in Table X. The eight mycelia of this table fall into four groups, reacting with one another in the same manner as did those of fruit-bodies *C* and *D* of Table IX.

Pairings were later made between the eight monosporous mycelia of fruit-body *E* and the two parent mycelia 7 and 25, as well as with other monosporous mycelia representing all the sexual groups in fruit-bodies

4 and 2. In this way, it was possible to make a fairly complete analysis of the sexual constitution of the hybrid fruit-body E. The results of the pairings are given in Table XI. Mycelia 81 and 85 have reacted with all of the mycelia from fruit-body 4, but with only mycelia 20 and 23 of fruit-body 2 ; they must, therefore, have the genetic constitution $A^2 B^2$, and are identical with parent mycelium 25. By comparing the reactions of mycelia 82 and 84 it will be seen that they must have the genetic constitution $A^4 B^4$, and are identical with parent mycelium 7. Mycelia 80 and 94 and 83 and 87 will be recognized as sex hybrids, having obtained one-half of their sex-factors from each parent. From the pairings with mycelia 47 and 58 of

E series	Myc.	A^2B^2 25	A^2B^2 26	a^2b^2 20	a^2b^2 23	A^2b^2 16	A^2b^2 29	a^2B^2 30	A^4B^4 7	a^4b^4 8	a^4b^4 5	A^4b^4 2	A^4b^4 11	a^4B^4 3	a^4B^4 9	a^3b^3 47	ab 58
A^2B^2	81	—	—	+	+	—	—	—	+	+	+	+	+	+	+	+	+
A^2B^2	85	—	—	+	+	—	—	—	+	+	+	+	+	+	+	+	+
A^4B^4	82	+	+	+	+	+	+	+	—	+	+	—	—	—	—	+	+
A^4B^4	84	+	+	+	+	+	+	+	—	+	+	—	—	—	—	+	+
A^2B^4	80	—	—	+	+	—	—	+	—	+	+	+	+	—	—	+	+
A^2B^4	94	—	—	+	+	—	—	+	—	+	+	+	+	—	—	+	+
A^4B^2	83	—	—	+	+	+	+	—	—	+	+	—	—	+	+	+	+
A^4B^2	87	—	—	+	+	+	+	—	—	+	+	—	—	+	+	+	+

TABLE XI. *Coprinus lagopus.* The pairing of eight monosporous mycelia from a first-generation fruit-body E of mycelium 25×7 with fourteen monosporous mycelia of the parent fruit-bodies No. 2 and No. 4, and one monosporous mycelium from each of fruit-bodies No. 3 and No. 1.

Tables III and I respectively, it is clear that the eight mycelia of fruit-body E have reacted like their parents with other sexual strains. During the development of the hybrid fruit-body, the segregation of the sex-factors must have taken place according to the theory of dihybridism, and no evidence has been obtained to show that mutations of sex-factors occurred. We may, therefore, conclude that when two monosporous mycelia of *Coprinus lagopus*, belonging to different sexual strains, unite to form a compound mycelium, a fruit-body arising from that mycelium will produce spores of four sexual groups ; 25 per cent. of the spores belong to group 1, and are of the same sex as one of the parents ; 25 per cent. belong to group 2, and are of the same sex as the other parent ; the remaining 50 per cent. belong to groups 3 and 4, and are hybrids deriving one sex-factor from each parent.

(5) *The Reduction Division and the Segregation of Sex-factors in Coprinus lagopus.*

On account of their small size, Kniep (6) was unable to isolate the four spores from individual basidia of fruit-bodies of *Schizophyllum commune*, but using *Aleurodiscus polygonius* he succeeded in obtaining the spores from thirty-five different basidia. When the four spores from each basidium were paired together, he found that in every case the spores from individual basidia were of two kinds only; i.e. if one pair had the factors (*A B*), the other pair had the factors (*a b*); while if one pair had the factors (*A b*), the other pair had the factors (*a B*). From this result, Kniep concluded that the reduction of sex-factors must have taken place with the *first* division of the fusion nucleus.

If, in *Aleurodiscus polygonius*, the reduction of sex-factors takes place with the first division of the fusion nucleus, no other result could have been obtained by Kniep. For example, if the fusion nucleus possesses the factors (*A B a b*), reduction and spore-formation will take place according to one of the following schemes:
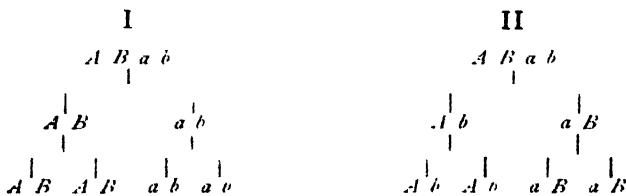
In either case each basidium would come to bear spores in pairs, of two kinds only.

A result similar to that recorded by Kniep might well have been obtained with reduction taking place with the *second* division of the fusion nucleus. Let us suppose, for example, that the fusion nucleus possessed the factors (*A B a b*); then reduction and spore-formation will take place in one or more of the following ways:
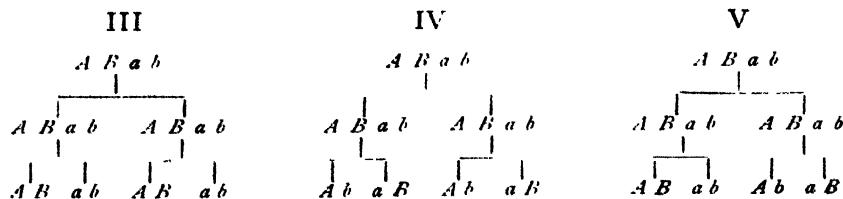
If the nuclear divisions were to take place as in case III or IV, each basidium would come to bear spores of two kinds only, and the pairing of spores from individual basidia would give results similar to those of

cases I and II, where reduction of the sex-factors takes place in the first division of the fusion nucleus. If, however, the divisions were to take place as indicated in case V, each basidium would come to bear four different kinds of spores. Therefore, although Kniep concludes that in *Aleurodiscus polygonius* reduction of the sex-factors takes place with the first division of the fusion nucleus, he might also have obtained the same result had reduction occurred in the second division. However, since Kniep made a study of thirty-five different basidia, all of which proved to be of the same type, the theory of probability justifies his conclusion that in *Aleurodiscus polygonius* the reduction of sex-factors takes place with the first division of the fusion nucleus.

By adopting the following new method (13) it was possible to secure the four spores from a number of basidia of fruit-bodies of *Coprinus lagopus*. From a young fruit-body which was just beginning to expand its pileus, a gill was removed and placed flat upon a glass slide. A cover-glass held by means of a small pair of forceps was then lowered gently until it touched as lightly as possible the upper surface of the gill ; it was then raised quickly. By examining the surface of the cover-glass under the microscope, it was found that in some places the tetrad from a single basidium had adhered to the cover-glass in the form of a minute square. The cover-glass was later fixed to a clean glass-slide by means of a little vaseline or Canada balsam, and the spores from each basidium were removed one at a time by means of the dry-needle method (3) and placed in hanging drops of dung-agar for germination. At the end of twenty-four hours, all viable spores had generally germinated, although spores which were not fully ripe when the gill was removed often germinated after two days. Considerable judgement must be used in selecting the gill at the proper stage of maturity ; if the gill is taken when too immature the spores will not germinate, while if left on the fruit-body until deliquescence has set in the securing of well-separated tetrads will be rendered extremely difficult. When germination of all four spores from any one basidium was found to have taken place, and the mycelia had developed sufficiently, transfers were made to plates of sterile dung-agar and, later on, the mycelia were paired together in the manner already described.

Using the above method, tetrads were obtained from eleven basidia, and, in addition, three spores were obtained from each of two other basidia. Six of the tetrads were taken from a fruit-body which had arisen from the union of mycelia 54 and 58 of Table I ; the remaining five tetrads, as well as the two groups of three spores, were taken from a fruit-body which had arisen from the union of mycelium 39 of Table III and mycelium 57 of Table I. Thus, the first fruit-body was the product of one sexual strain, while the second was the product of two sexual strains. The results of the pairings brought out the fact that in both fruit-bodies two kinds of

basidia were present; the first type, shown in Table XII, is similar to that shown by Kniep for *Aleurodiscus polygonius,* having only two kinds of spores; the second type, shown in Table XIII, has *spores of four kinds.* Of the 13 basidia studied, 7 were of the first type, and 6 were of the second type. Since a reduction in the second division of the fusion nucleus would result in both types of basidia being present, we are justified in concluding that in *Coprinus lagopus* reduction of the sex-factors takes place in the *second* division of the fusion nucleus.

In the preceding study of the basidia of *Coprinus lagopus* pairings were made only between the four spores of each individual basidium. In order

TABLE XII. *Coprinus lagopus.* All possible pairings of four monosporous mycelia from a basidium of fruit-body 54 × 58; spores of two kinds.

TABLE XIII. *Coprinus lagopus.* All possible pairings of four monosporous mycelia from a basidium of fruit-body 54 × 58; spores of four kinds.

to determine the reaction which the spores from any one basidium will give when paired with the spores from a number of other basidia, all possible pairings were made between the spores from five basidia of fruit-body 39 × 57; the results are embodied in Table XIV. In the whole table only four sexual groups are represented. Basidia 2, 3, 5, and 7 have proved to be alike, each one having spores of four kinds; in fact, any two of these basidia might be interchanged without altering in any way the appearance of the table. Basidium 6 has spores belonging to only two of the sexual groups, but both of these groups are already represented by two of the spores from each of the other four basidia. By obtaining the four spores from a single basidium of the type shown in Table XIII it is, therefore, possible to obtain one representative of each of the four sexual groups found for *Coprinus lagopus.*

On theoretical grounds it might be expected that of the basidia bearing two kinds of spores in pairs some would be of the type (*A B*) (*A B*) (*a b*) (*a b*), while others would be of the type (*A b*) (*A b*) (*a B*) (*a B*). A reference to the diagrams for cases III and IV (p. 447) will make this

point clear. Little difficulty should be experienced in determining whether or not the two types of basidia actually occur in nature. If pairings were made between monosporous mycelia from a number of basidia bearing spores of two kinds in pairs, the presence of two types of basidia would readily become apparent, since monosporous mycelia from a basidium of

TABLE XIV. *Coprinus lagopus.* All possible pairings of twenty monosporous mycelia from five basidia of fruit-body 39 × 57.

type (*A B*) (*A B*) (*a b*) (*a b*) would fail to react with those from a basidium of type (*A b*) (*A b*) (*a B*) (*a B*).

Kniep (6), in his study of the basidia of *Aleurodiscus polygonius*, observed that frequently two of the spores from a basidium germinated and developed mycelia more rapidly than the remaining pair. When pairings of the monosporous mycelia were later made, the two spores which had germinated first were found to belong to the same sexual group, e. g. (*A B*), while the two which had germinated tardily were found to belong to another sexual group, e.g. (*a b*). In the present study of *Coprinus lagopus*, no correlation whatever has been observed between the sexual reaction and

the manner of germination of the spores and the growth of the mycelia. When grown on plates of sterile dung-agar, individual monosporous mycelia of *Coprinus lagopus* exhibit marked differences in rate and habit of growth and in the production of oidia. Some cultures produce oidia very sparingly, while others produce an abundance of oidia distributed evenly over the surface of the agar; in many of the cultures the oidia appear in clearly marked concentric rings exhibiting an apparent diurnal periodicity. In two of the basidia produced by fruit-body 54 × 58, distinct differences in the rate of spore germination and mycelial growth were observed. Two of the spores from each basidium had germinated at the end of twenty-four hours, and the resulting mycelia grew rapidly and produced an abundance of oidia; the remaining two spores from each basidium required over forty-eight hours for germination, while the mycelia produced few oidia and grew so slowly that at the end of three weeks the growth upon each agar plate was only about 5 cm. in diameter. When the pairings were made, one of the basidia proved to be of the type shown in Table XIII, with spores belonging to the four sexual groups; the other basidium was of the type shown in Table XII, with only two of the groups represented, but the two spores of slow growth belonged, not to the same group as might have been expected, but to different groups. In *Coprinus lagopus*, therefore, no evidence has been found to show that the manner of spore germination and rate of mycelial growth are in any way correlated with the segregation of sex-factors.

(6) *The Fruiting of Monosporous Cultures of Coprinus lagopus.*

Mlle Bensaude (1), working with *Coprinus fimetarius*, concluded that in a heterothallic species monosporous mycelia are always sterile. Since she made observations on only two cultures, the evidence which she obtained is not wholly conclusive. As already stated, Kniep (5) obtained a fruit-body from a monosporous mycelium of *Schizophyllum commune*, but the spores produced by this fruit-body were all of one sex. Vandendries (11) states that one of his monosporous cultures of *Panaeolus campanulatus* produced a fruit-body, but that the spores from it failed to germinate. In her second paper, Miss Mounce (10) records that she observed marked differences in the fruiting power of monosporous and polysporous mycelia of *Coprinus lagopus*. In the present study, upwards of eighty monosporous mycelia of *Coprinus lagopus* were grown in tubes of sterile horse-dung three inches long and nine-tenths of an inch in diameter; these cultures provided very suitable material for a study of the fruiting power of monosporous mycelia. All of these mycelia produced fruit-bodies, but with one exception, that of mycelium 53, which will be referred to later, the fruit-bodies produced were distinctly abnormal in appearance. Fruit-bodies arising on the bisporous

or polysporous mycelia having clamp-connexions were invariably few in number, generally not more than two or three to each tube culture, but all of these fruit-bodies elongated their stripes and shed an abundance of black spores in a normal manner. The monosporous fruit-bodies, on the contrary, were relatively much more numerous, each tube culture producing from eight to ten ; some of these fruit-bodies remained as small rudiments, others developed pilei without maturing any spores or elongating their stipes, while still others elongated their stipes and produced a few ripe spores, but normal spore discharge was never observed. In all cases the pilei of the monosporous fruit-bodies remained white or pale grey in colour, owing to the small number or entire absence of ripe spores on the gills, in contrast with the black colour so characteristic of the normal secondary fruit-bodies.

Spores were obtained from a monosporous fruit-body of mycelium 1 of Table IV by touching a sterile platinum loop to the gills. The spores were then placed to germinate in five hanging drops of dung-agar, each drop containing about 1co spores. At the same time, spores from a normal secondary fruit-body (fruit-body 2 of Table II) were placed in two similar hanging drops. At the end of six hours, some of the spores from both fruit-bodies had started to germinate; at the end of twenty-four hours, about 85 per cent. of the spores in each of the seven hanging drops had germinated. The mycelia in all of the drops seemed to grow at the same rate, and all produced an abundance of the characteristic oidia. When examined seven days later, the mycelia from spores of the secondary fruit-body showed numerous clamp-connexions and had ceased producing oidia. while those from spores of the monosporous fruit-body had no clamp-connexions, but continued to produce oidia. Thus, we must conclude with Kniep that the spores from a monosporous fruit-body are all of one sex.

About 500 spores from the monosporous fruit-body referred to in the last paragraph were sown in polysporous culture, and the resulting mycelium was paired with the parent mycelium 1, and also with ten other monosporous mycelia derived from the same fruit-body as mycelium 1. The results of these pairings, given in Table IV under the column M 1, show that the spores from the monosporous fruit-body have the same sex as that of the parent.

Later, a portion of the polysporous mycelium M 1 was transferred to a tube of sterile dung. Fourteen days from the date of the transfer, a number of rudimentary fruit-bodies appeared on the surface of the dung, but only one of these fruit-bodies developed further. Four days later, this fruit-body elongated its stipe and shed a very few spores. A culture from the interior of the stipe of this fruit-body was made on a plate of sterile dung-agar, but no clamp-connexions ever developed. Spores were placed to germinate in two hanging drops of dung-agar, as well as in a plate of sterile dung-agar, each culture containing about 500 spores. Germination took place readily, but no clamp-connexions ever developed.

One of these polysporous cultures was then used for pairing with the eleven monosporous mycelia of Table IV and the polysporous mycelium *M* 1. From the results of the pairings given in Table IV (p. 437) under the column *M* 2, it will be seen that the polysporous mycelium has reacted in all respects like the original parent, mycelium 1. A portion of the polysporous culture *M* 2, when later transferred to a tube of sterile dung, gave rise to three fruit-bodies. These fruit-bodies elongated their stipes, but the pilei were pale grey in appearance and did not possess the normal number of spores. Unfortunately it was not found possible to make a study of these fruit-bodies.[1] Imperfect fruit-bodies of *Coprinus lagopus* have, therefore, been obtained from three successive generations of primary mycelia which originated from a single spore, the first two generations producing spores all of one sex, the sex being identical with that of the original parent mycelium.

It sometimes happens that secondary or diploid mycelia give rise to imperfect fruit-bodies similar in appearance to those produced by primary or haploid mycelia. A description of these imperfect fruit-bodies, with illustrations, will be found in Buller's 'Researches on Fungi', vols. ii and iii. As fruit-bodies of this kind might conceivably arise from hyphae of the compound mycelium which had not yet developed clamp-connexions, a portion of the interior of the stipe from an imperfect fruit-body was removed with a sterile scalpel and placed on a plate of sterile dung-agar in order to determine the condition of the mycelium growing out from it. When examined six days later, the mycelium was seen to be producing an abundance of clamp-connexions, thus proving that the imperfect fruit-body had arisen not from primary but from secondary mycelium.

From the above observations we may conclude that the primary or haploid condition of the mycelium of *Coprinus lagopus* is associated with the production of imperfect fruit-bodies ranging in size from tiny rudiments to almost perfect fruit-bodies, the spores of which are of only one sex, identical with that of the parent, while the secondary or diploid condition of the mycelium, although occasionally giving rise to imperfect fruit-bodies, is usually associated with the production of perfect fruit-bodies having spores of the four sexual groups.

(7) *The Abnormal Behaviour of certain Monosporous Mycelia of Coprinus lagopus.*

Kniep (5) states that when certain monosporous mycelia of *Schizophyllum commune* were kept in pure culture for a long time they developed clamp-connexions, thus passing definitely from the haploid to the diploid

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[1] Four months later, spores from these fruit-bodies were sown in polysporous culture. The resulting mycelium remained primary, but produced two imperfect fruit-bodies (fourth generation), the spores of which, when sown in polysporous culture, produced only primary mycelium.

condition. Results analogous to these have been obtained with three monosporous mycelia of *Coprinus lagopus*, Nos. 26, 87, and 53.

After the work so far recorded in this paper had been completed, the various cultures of monosporous mycelia about to be discarded were given a final examination. It was then seen that mycelia 26 and 87 had small patches of hyphae bearing clamp-connexions. About twelve days prior to the time of this examination, a sub-culture of mycelium 26 had been made in a shallow glass bottle plugged with cotton-wool. A transfer was, therefore, made from this bottle to a plate of sterile dung-agar. When examined six days later, the mycelium in the plate culture was found to be in the primary condition and producing rings of oidia, showing no clamp-connexions whatever. A sub-culture of mycelium 87 had not been made, but on the date of the last transfer, about a week before the final examination, this mycelium was still in the primary condition.

After mycelium 53 had been used for pairing with other mycelia, it was sub-cultured in a tube of sterile horse-dung. About two weeks later, this mycelium gave rise to eight small fruit-bodies which at first were similar in appearance to the imperfect fruit-bodies produced by monosporous mycelia. Later, however, the eight fruit-bodies elongated their stipes, expanded their pilei, and shed an abundance of spores in a quite normal manner. When a portion of the mycelium from the dung tube was examined under the microscope, it was seen to possess clamp-connexions. Two sub-cultures of mycelium 53, previously made on plates of sterile dung-agar, were then examined and found to be still in the primary condition : moreover, sub-cultures from one of these plates remained in the primary condition for over a month longer and were only discarded at the conclusion of the work. In view of the fact that eight perfect fruit-bodies had been produced from a secondary mycelium on so small an amount of dung, while two sub-cultures of the same mycelium on dung-agar had remained in the primary condition, it seemed probable that mycelium 53 had changed spontaneously from the primary to the secondary condition. Spore-deposits were collected, therefore, from three of the fruit-bodies for the purpose of studying any abnormal changes which might have taken place. Unfortunately, only a short time could be devoted to this work, and the study is, therefore, very incomplete. Monosporous mycelia, however, were isolated from two of the fruit-bodies, five from fruit-body *F* and five from fruit-body *G*, and all possible pairings were made between them. The results of the pairings are given in Table XV. An examination of this table will show that spores of only three sexual groups have been obtained from each fruit-body, but spore *G* 4 is similar to spores *F* 4 and *F* 5, and spore *F* 2 is similar to spores *G* 1 and *G* 5, so that in the ten spores there are four sexual groups represented. It is probable that with a larger number of monosporous mycelia the four groups would have been found in each fruit-body.

The most striking part of the table lies in the reactions which have taken place between the different groups. Groups 1 and 2 have reacted together normally, as have also groups 3 and 4, but group 3 has reacted also with groups 1 and 2 ; in fact mycelia $F1$ and $F3$ exhibit the phenomenon of complete fertility with the other eight mycelia and conduct themselves in all respects like mycelia from a new sexual strain. One other point of interest in this table is the reaction which has taken place between mycelia $G2$ and $G3$, which belong to the same group.

From the preceding evidence it would be unwise to conclude that

		1			2			3		4	
		F4	F5	G4	F2	G1	G5	F1	F3	G2	G3
1	F4	—	—	—	+	+	+	+	+	—	—
	F5	—	—	—	+	+	+	+	+	—	—
	G4	—	—	—	+	+	+	+	+	—	—
2	F2	+	+	+	—	—	—	+	+	—	—
	G1	+	+	+	—	—	—	+	+	—	—
	G5	+	+	+	—	—	—	+	+	—	—
3	F1	+	+	+	+	+	+	—	—	+	+
	F3	+	+	+	+	+	+	—	—	+	+
4	G2	—	—	—	—	—	—	+	+	—	+
	G3	—	—	—	—	—	—	+	+	+	—

TABLE XV. *Coprinus lagopus.* All possible pairings of ten monosporous mycelia from fruit-bodies *F* and *G* of mycelium No. 53.

mycelia 26 and 87 of *Coprinus lagopus* suddenly became secondary in a spontaneous manner, since there is always the possibility that where so many cultures were being made contamination may have taken place from outside sources, not so much by means of spores as from oidia which may have been set free in the air. Such a possibility, however, cannot account for the abnormal behaviour of mycelium 53; the large number of fruit-bodies produced by this culture, together with the striking character of the reactions between monosporous mycelia from two of these fruit-bodies, point to the conclusion that this mycelium suddenly mutated from the primary to the secondary condition, and in so doing gave rise to a fruit-body having some spores of a new sexual strain. Mutations of this character may indeed account for the many different sexual strains of *Coprinus lagopus* which are to be found among wild fruit-bodies.

SUMMARY.

1. A study has been made of the sex of *Coprinus lagopus*. The fruit-bodies employed in this work were collected from four widely separated points in Canada, and from three places at Birmingham, England.

2. All strains of *Coprinus lagopus* studied proved to be heterothallic. Miss Mounce's suggestion that there may exist both homothallic and heterothallic strains of this fungus has not been supported.

3. English and Canadian strains of *Coprinus lagopus* are similar morphologically and have been shown by the clamp-connexion criterion to be identical.

4. Sex in *Coprinus lagopus* is determined by certain factors which segregate out according to Mendelian principles.

5. Monosporous mycelia from any individual fruit-body of *Coprinus lagopus* belong to four sexual groups similar to those found by Kniep for *Schizophyllum commune*.

6. Complete fertility results when monosporous mycelia from wild fruit-bodies of different sexual strains are paired together.

7. Two fruit-bodies from a secondary bisporous mycelium produce spores belonging to but four sexual groups, the same four groups being present in both fruit-bodies.

8. Monosporous mycelia from a first-generation hybrid fruit-body react with the parent mycelia strictly in accordance with the theory of dihybridism.

9. The reduction of sex-factors in *Coprinus lagopus* takes place with the second division of the fusion nucleus, resulting in the production of (*a*) basidia each with spores of only two kinds, and (*b*) basidia each with spores of four kinds.

10. No correlation has been found between the rapidity of germination of spores from individual basidia and the segregation of sex factors, as observed by Kniep for *Aleurodiscus polygonius*.

11. The primary or haploid condition of the mycelium was found to be associated with the production of imperfect fruit-bodies bearing spores of only one sex, the sex being identical with that of the parent mycelium ; the secondary or diploid condition of the mycelium was found to be associated with the production of perfect fruit-bodies bearing spores of the four sexual groups.

12. Imperfect fruit-bodies were produced by three successive generations of primary mycelia originating from a single spore ; fruit-bodies of the first two generations had spores of only one sex, the sex being identical with that of the parent mycelium.

13. The imperfect fruit-bodies which are sometimes produced by

secondary mycelia arise not from primary, but from secondary strands of mycelium.

14. One monosporous mycelium appears to have mutated from the primary to the secondary condition, and in so doing it gave rise to a fruit-body having some spores of a new sexual strain.

15. A method is described for removing the four spores from a living basidium, separating them from one another, and placing each one of them in a separate hanging drop of dung-agar.

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The Regional and Seasonal Distribution of Potassium in Plant Tissues.

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With Plate XII and six Figures in the Text.

CONTENTS.

1. INTRODUCTION.

THE distribution and seasonal variation of certain elements essential for growth of the organism is a matter of great interest, and the present paper is an attempt to apply microchemical methods of investigation to the presence of potassium in the tissues of a few plants. The technique used is that described by Macallum (1). This method was first used by Macallum on animal tissues, but he extended its use to various plant tissues, and the general conclusions he arrived at may be briefly summarized as follows:

H h 2

1. ' As revealed by the method, potassium occurs in both the cytoplasm and the intracellular structures. In the latter it is present as a product of impregnation and infiltration, and as a consequence there are few such structures that are free from it. . . . The potassium obtaining in cytoplasm occurs in two conditions, that of physiological precipitation and that of physiological or biochemical condensation.'

2. ' The cell nucleus does not normally contain the slightest trace of potassium.'

3. ' The facts so far ascertained point definitely to a participation in some way of potassium salts in the processes of assimilation. . . .'

4. ' In the growth and formation of vegetable cell-walls, potassium seems to play a part.'

2. METHOD.

The method for demonstrating potassium in cells consists in the use of the hexanitrite of cobalt and sodium, which in the presence of sodium acetate gives an immediate precipitation for potassium. This precipitate is rendered more visible by ammonium sulphide, when it appears as a dense black precipitate. The tissues are left in the cobalt reagent for a minute or two and then washed for twenty to thirty minutes in a succession of basins of ice-cold water which are kept over a freezing bath. The sections can then be mounted in glycerin and fresh ammonium sulphide. In the course of the investigation, particular attention has been paid to the reliability of the method and to the possibility of diffusion before the penetration of the reagent. A method has been devised by which the material is instantaneously frozen by solid carbon dioxide, remaining frozen during the cutting process and only thawing after being placed in the reagent. For the suggestion of this method the author is indebted to Dr. J. B. Collip.

The process consists in cutting the sections with a Spencer carbon dioxide freezing microtome kept within a glass-topped box, so that thawing of the sections while being removed from razor to reagent can be prevented by means of a carbon dioxide spray, which keeps the air and razor within the box below freezing-point. The microtome and sections are manipulated through canvas arm-holes in the side of the box.

During the earlier stages of the work a long series of observations were carried out to determine whether this method eliminated diffusion. Hand sections and microtome sections were cut without freezing, the subsequent treatment being the same as that outlined above. In all these cases there was obvious diffusion of potassium without the cytoplasmic structures of the cell, often extending into the mounting medium about the section. Comparison was made of the same tissues by the two methods, and in all cases the contrast in the appearance was very marked. In the unfrozen material

the distribution of the potassium was indefinite, diffused strands of potassium giving a smeary appearance. In the frozen material the position of the potassium was definite and clear-cut, with no diffusion without the cell. Furthermore, there was a remarkable constancy in the position of the potassium in successive microtome sections of the same tissue.

With this method the only possibility of the rearrangement of the potassium within the cell would be at the instant of freezing or at the moment of penetration of the reagent into the thawing cells. The freezing may be taken as practically instantaneous at a temperature of $- 50°$ C.; the only possible rearrangement being the precipitation of the excess potassium thrown out of solution.

This method was fully tested on the following plants: *Spirogyra, Draparnaldia, Allium, Lilium, Phaseolus, Tradescantia, Ficus, Pyrola,* and *Solanum,* while the distribution of potassium in the spruce and in the germinating wheat grain was studied in detail.

3. Criticisms of Method.

The reagent has been criticized by certain workers as being unreliable, but any variation in the composition of the precipitate is due to the amount of sodium entering into it, so that although the reagent may be less suitable for quantitative analysis, this does not affect it as a microchemical reagent.

Molisch (2) states that ammonia reacts with the reagent similarly to the potassium. Although Macallum says that the ammonium salts so formed dissolve away readily in ice-cold water, so that they do not complicate the reaction, my results do not confirm this.

A spruce shoot previously placed in ammonium chloride solution for some hours when treated appropriately for potassium showed a very dense precipitate in the tracheides. Spruce growing in water as a control when similarly treated gave scarcely any precipitate in the tracheides, so that the reaction in the first case must have been due to ammonia. Although the ammonium compound is more soluble than the potassium, yet even when sections are washed for one and a half hours the black ammonium precipitate is not completely washed out.

The strong toxic action of ammonia would prevent it from being present in very large quantities in the plant, in contrast with potassium, which is a necessary constituent for growth. Indeed, a water extract of spruce leaves when treated with Nessler's solution showed no test for ammonia, although the proteins present produced a yellow coloration, changing to red on standing. When these proteins and certain acids were coagulated by lead acetate solution, the filtrate gave no appreciable ammonia reaction, although Nessler's test is an extremely delicate one.

On the other hand, when the ash of such leaves was treated for potas-

sium by the potassium chloroplatinate method the yield from 10 grm. of fresh spruce leaves was 18 mg. of potassium. This reading was made in the late autumn, when potassium is comparatively poor in the leaves.

The wheat grain also contains no ammonia, so that there is no possibility of the precipitate obtained in the examination of either of these plants being anything other than potassium.

The possibility of obtaining additional information on the distribution of potassium by means of this reagent was drawn to my attention by Dr. F. J. Lewis, and I am indebted to him for suggestions and criticisms during the progress of the work.

4. EXAMINATION OF THE POTASSIUM CONTENT OF *PICEA CANADENSIS*, (Mill) B. S. P.

A. *Material used.*

During the months of November, January, and February a systematic examination of the tissues of the White Spruce was carried out. The material selected was (*a*) spruce in its natural habitat in the valley of the Saskatchewan River ; (*b*) small spruce about five years old, potted in large wooden boxes in August and brought into the greenhouse in September. The first material was exposed to the usual low winter temperatures of this country, while the second was kept at a temperature of 60–65° F. during the whole period. Observations were made on outdoor material up to the end of April to ascertain whether any changes in distribution were associated with the beginning of spring growth.

B. *The Mature Root.*

The first material to be examined was taken from roots about five years old growing in their natural habitat.

Fig. 1, Pl. XII, shows diagrammatically the relative richness in potassium of the various tissues as shown in a cross-section of the root.

The Conducting Tissue. Except for a few grains in the lignified parenchyma at the centre of the woody cylinder, the xylem of the root contains no potassium. This poverty of potassium within the tracheae is general throughout the spruce.

The medullary rays running through the wood contain a fair amount, especially round the pits, but these rays become much richer as they traverse the phloem. The part of the vascular tissue showing the most marked reaction is the cambium, where the cell cavities are black with large granules ; in fact it has been found that meristematic cells are usually rich in potassium. The mineral is in moderate quantities in the first-formed phloem, increasing in amount towards the cambium. Also the marginal and secretory cells

round the resin ducts—whether these ducts occur within or without the vascular tissue—are comparatively rich.

Outer Tissues. The pericyclic cells show marked potassium contents, which occur as granules precipitated throughout the cytoplasm. Neither the phellogen nor the phellem of the root contain any potassium, but the cavities of the phelloderm are lined with large-sized grains.

When these roots were compared with material from the greenhouse in January, little difference was noticed except a very slight increase of potassium in the tracheides of the latter over those from outside. Roots from outside were examined at the end of April, and then the tracheides showed a much greater increase in potassium content.

C. *The Finer Rootlets.*

In the months of November and February, longitudinal sections were taken of the finer roots with the absorbing rootlets attached, and a marked variation was found in the potassium content as compared with the older roots.

The tracheides of the rootlets were rich in potassium, and this was invariably collected around the bordered pits. The phloem and pericycle contained even more potassium than the old roots, while in the cortex, a tissue that is lost in later life, there was scarcely any.

It is possible that the potassium absorbed by the rootlet is translocated by way of the conducting strands of the young roots, and then stored in the pericycle of the older roots.

D. *The Root-tip.*

During the winter the apex of the root-tip shows a white dome of tissue, about 1 mm. long, emerging from the corky covering of the older portion of the root. In greenhouse material these apices begin to elongate in February at the same time as the opening of the buds. This new growth does not become covered with cork until late in the season. In outside material the elongation of the root-tips does not take place until May, when they appear as shown in Text-fig. 1.

When the root-tips from the spruce growing outside in February were sectionized and tested for potassium, it was found the meristematic cells contained only a few very fine granules.

The elongating roots from the greenhouse spruce, however, showed, when tested for potassium, a much more marked reaction in the meristem. Potassium seems to be translocated to the meristem during elongation for the formation of new tissues, the same thing being observed in the stem apex.

Priestley and Tupper-Carey, in their work on the impermeability of root-tips (3), say: ' In experiments with electrolytes, in which the distribution of the kations can be subsequently examined by characteristic colour reactions, no cases have been noted where the kations could be detected in the meristematic cells. . . . Thus in healthy willow roots the distribution of potassium followed by Macallum's sodium nitrite reaction . . . showed a sharp restriction to the vacuolated region behind the meristematic apex.'

I have not found this generalization to hold for any of the plants examined, although in the apex of the spruce root there is very little potassium present during the resting period. The fluctuations observed in
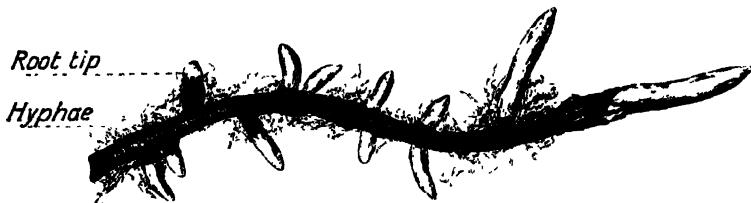
TEXT-FIG. 1. *Picea* root, showing elongating apices. Greenhouse material collected February 23, 1924.

this plant seem to show that there is a translocation of potassium down to the growing-point in the spring.

Small roots were immersed whole in the reagent to test the white mat of hyphae that envelops the root. It was found that these hyphae contain only traces of potassium, quite irregularly distributed.

E. *The Stem.*

Text-fig. 2, Pl. XII, illustrates the relative amount of potassium in the stem tissues.

In the pith the cavities of the majority of the parenchyma cells show a very slight reaction, except for certain cells peculiarly rich in potassium, which are scattered throughout the pith of the first-year shoot. They are shown in transverse section to be special cells arranged singly or in groups, particularly near the vascular strands, and in longitudinal section a cylinder of these ' potassium cells' appears along the outer margin of the pith, and seems to connect up with the medullary rays. The bars of stone cells that occur across the pith are empty in the older twigs, but potassium is present in the majority of these cells towards the bud.

The only trace of potassium in the tracheides consists of a few grains on the bordered pits, though the protoxylem is somewhat richer. The tracheides had been so constantly found poor in potassium that it was sus-

pected that the contents might have diffused out into the reagent, because a solution would not be held within the cavities of the tracheides as easily as a precipitate would in the cytoplasm of a living cell. To test this, twigs were kept for a day in a saturated solution of potassium chloride and then cut. It was found that the tracheides gave a very good reaction, so that when the conduction-stream is rich in potassium the methods used here would show it.

The cambium and phloem are fairly rich, the potassium in the phloem occurring in a coarser precipitation than that in the cambium. The medullary rays show a marked reaction, especially in the pits.

The cells of the cortical parenchyma show only minute granules in an amount that varies in different cells. The marginal cells of the resin-ducts show the same richness in potassium as they do in the root. Although many of the phellem cavities are empty, large localizations of potassium occur irregularly in groups of cells. The phellogen, the scelerenchyma, and the epidermal cells are rich in potassium.

Cells of the same appearance as those described above in the root as phloem parenchyma appear irregularly in the pith, phloem, and cortex of the stem. When sections are stained in haematoxylin and safranin, the contents of these cells appear bright red. They are peculiar in that the protoplasm is in a coarser reticulation than in other cells. They contain a sprinkling of potassium.

In comparing the February material from the open and from the greenhouse, it was found that the protected trees were richer in potassium in the wood and in the cortex, obviously due to the increased flow of sap. The localization referred to in the periderm seems often to be a result of low temperatures and a consequent ' salting out ' of compounds in the cell, for no localization of potassium can be noticed in the periderm of the green-house material, but the mineral occurs scattered in the cavities as small grains.

Material examined at the end of April showed an increase in the amount of potassium in the xylem over that found in the winter months.

F. *The Apical Bud.*

The diagramatic representation of the distribution of potassium as it appears in a longitudinal section is shown in Fig. 3, Pl. XII. The bud had been taken in November from a tree growing in its natural habitat.

A previous paper (4) explains the structure of the bud in detail. It has been found that the embryonic cone is borne on a plate of rigid thick-walled tissue, beneath which the pith breaks down to form a cavity. The cone of meristematic tissue bearing the embryonic leaves has been found to be very rich in potassium. Each cell is so crowded with black grains that

no difference in content in the various tissues of this region can be seen, all cells appearing uniformly dark. Also, the cavities of the 'crown' below are turned quite black with the reagent, showing a particularly strong reaction in the region towards the cone.

The appearance of the 'crown' cells when treated for potassium is shown in Text-fig. 2. The cavities of the cells are rich in the mineral, and there are also potassium markings on the cell-wall.

On examining sections of the unstained tissue, parallel channels like very long pits appear, running through the crown. When treated with the
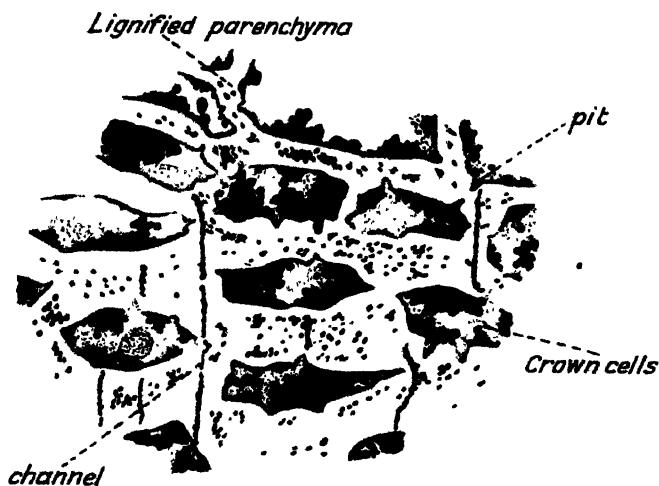
TEXT-FIG. 2. Potassium in the 'crown' cells of the Spruce bud, November 13, 1923.

cobalt reagent they turn black with potassium, as is shown in Text-fig. 2. Other substances than potassium may possibly use this path in travelling to the bud.

In order to determine the subsequent distribution of potassium in the meristematic cone after elongation, buds in their winter condition were put in a solution of dextrose, which seems to promote the growth of the buds more quickly than water, the new leaves then appearing in about three weeks. The observations so obtained have been confirmed by an examination of the elongating bud growing outside in April.

Longitudinal sections of this material show that the elongated meristematic cone is no longer uniformly rich in potassium, but that there has evidently been translocation, chiefly to the tip of the cone just above the second layer of 'crown' cells that have begun to develop. It was also localized to the sides of the cone, in the procambial strands, the young cortex, and the embryonic leaves. The plerome is differentiated as it takes

up a brown colour due to the nitric acid in the reagent; it is poor in potassium. From this distribution it seems that potassium is connected with the growth of new organs, for there is always abundance both in the stem apex and in the embryonic leaves.

It was found that when the buds with the scales removed were immersed whole in the cobalt reagent, no penetration took place in spite of the remarkable diffusibility of the solution. This was partly due to the cutin that surrounded the embryonic leaves, but as the detached portion of the bud was in contact with the reagent, it was concluded that this tissue also offered resistance to diffusion. To test this, some buds were dissected from the twig at the level of the cavity, so leaving the crown to protect the base of the cone. These were compared with buds from which the plate of crown cells had been dissected off. When the whole bud with the crown attached was immersed in the reagent for one hour, there was only a slight coloration just above the crown. On the other hand, in the bud from which the crown had been removed, the reagent penetrated to the leaf margins in a few minutes.

It is interesting to notice that this relative impermeability can be removed, for after treatment with ether or boiling in water the walls of the 'crown' cells readily allow the penetration of the reagent.

Observations were made on the structure of the nodes some distance down the stem, in order to examine any changes subsequent to the elongation of the bud in those tissues abutting on the cavity. In the first place, it was found that the pith in the region of the nodes is composed of round cells that are quite rich in potassium, in contrast with the square-shaped cells that make up the pith in the internodal region, and which only yield a slight reaction. At the base of the cavity is a layer of broken-down tissue. Above the cavity is the persistent crown, which can be seen to be composed of two types of cells. Bordering the cavity are cells with slit-like lumens, and above them is a tissue, probably arisen from the pith, with square cavities and lignified walls. The former is poorer in potassium than the latter.

These two types of cells in the crown can also be seen, though not so markedly, in the bud, and are illustrated in Text-fig. 2.

G. *The Leaves.*

Leaves were first examined during November from trees growing in their natural habitat. As regards the vascular system of the leaf, the tracheides contain but little potassium except for a few grains collected round the bordered pits, while the transfusion tracheides show no reaction, these elements in the leaf thus agreeing with the scanty distribution of potassium in the xylem of all parts of the tree.

The potassium in the medullary rays reaches a maximum in the

bordered pits leading from the rays to the tracheides. The phloem is rich in potassium; in the albuminous cells there is a slight precipitate. The sclerenchyma, like most of the non-living elements, is empty, while the endodermis contains a varying amount.

It might be expected that the distribution and relative amount of potassium in the chlorophyll cells would suggest some connexion with this substance and the activity of the chlorophyll apparatus. Further, there are marked features of seasonal change both in the condition of the chlorophyll and in its position in the cell as described by Lewis and Tuttle (5). With this object in view, the mesophyll cells from leaves outside and from the experimental plants in the greenhouse were examined, and the relative condition of these are shown in Figs. 4 and 6, Pl. XII, while Fig. 5, Pl. XII, a drawing of the mesophyll in the spring, shows an intermediate condition.

In the outside material, illustrated in Fig. 4, Pl. XII, we see that large amounts of potassium are present in the cell in the form of extensive areas, obviously related to the ' laked ' chloroplasts and finer sheets which usually occur in the neighbourhood of the cell-wall. In general, it may be said that the potassium shows a marked tendency to occur in clumps in these leaves, while in the leaves from the greenhouse material there is a tendency to form fine reticulations about the chloroplasts.

Fig. 5, Pl. XII, is a drawing of a mesophyll cell in spring condition, showing the migration of the chloroplasts away from the nucleus previous to their taking up the position shown in Fig. 6, Pl. XII. Although the potassium is still collected round the nucleus, it also is tending to spread to the periphery of the cell in the same manner as the chloroplasts. In the same material cells can be seen with the chloroplasts in summer condition, and in these cells the potassium is evenly distributed throughout the cell. This same association between chlorophyll and potassium, clearly shown in Fig. 6, Pl. XII, appears to be a constant feature, and has been noticed by Macallum and by the author in *Draparnaldia* and *Spirogyra*.

The network of potassium between the chloroplasts, as seen in the summer, may be explained as an adhesion phenomenon.

In the winter, when the chloroplasts are collected closely about the nucleus, the potassium grains are usnally collected together near the chlorophyll. It has been suggested that such localizations do not represent the actual distribution of the mineral, but that they are the result of secondary changes subsequent to the addition of the reagent. This possibility must be borne in mind, but because of the constancy with which the potassium within the cell takes up a certain form at each season, a second explanation may be considered.

According to Wolff's table of ash analysis (6) the fir contains nearly 3 per cent. of potassium, and it is probably more concentrated in the spruce leaf. Though it takes 60 grm. of potassium chloride at 104° C. to saturate

1co c.c. of water, at 0° C. any excess over 3 grm. will be precipitated. The low winter temperatures of this region may throw excess potassium out of solution in the cell, resulting in a different appearance upon microchemical analysis. In support of this view, it may be noted that localizations of potassium occur in the periderm of the spruce stem in the winter, but not in the summer.

Localizations also occur at higher temperatures in certain plants that are particularly rich in potassium, as in the epidermal cells of onion and hyacinth leaves. In regard to these plants an interesting feature may be referred to. On examining the stomata it was found that potassium occurred in large masses in the corners of the mature guard-cells, where the oil and starch can be found, but in the initial cells not yet divided the potassium was scattered in small granules in the cytoplasm, with no hint of any localizations.

An examination of spruce leaves during the year shows that from November to February there is a falling off in the content of the mesophyll cells and a corresponding increase within the vascular tissue. This suggests that in the latter part of the winter potassium is translocated away from the leaves by way of the conducting strand, possibly to be used by the developing bud in the spring.

5. EXAMINATION OF POTASSIUM CONTENT IN THE WHEAT GRAIN.

With the object of obtaining further evidence regarding the distribution of potassium salts in meristematic tissues, it was decided to make a detailed study of potassium in germinating wheat. The varieties examined were: 'Taylor's Wonder Canadian Spring Wheat', 'Marquis Spring Wheat', and 'John Bull English Winter Wheat'.

In Text-fig. 3 is shown the distribution of potassium in a section of a wheat grain after germination for two days in distilled water.

A. *The Pericarp and Endosperm.*

On studying the various tissues, it was found that the outermost layers give a faint reaction, the epidermis being fairly rich and the pericarp poor. The wall of the old seed-coat is impregnated with potassium markings on its surface, an appearance that has also been noted in the walls of the epidermal cells from the onion bulb and in the 'crown' cells of the spruce bud.

Text-fig. 4 shows the occurrence of potassium in the outer layers of the grain. It can be seen that the aleurone layer gives a very dense reaction, the cavities being crowded with granules. In the endosperm, however, there is only the slightest trace of potassium, and that occurs as fine

particles scattered amongst the starch grains. The contrast between this condition and that of the aleurone layer is very striking, and there is no evidence of any transference of potassium from the aleurone layer into the endosperm during germination.

When 'John Bull' wheat was tested, after germinating for fifteen days, no tissue, apart from the embryo, showed any reaction. Even the aleurone layer was quite empty. When a tissue is dead it is to be expected that the potassium salts should disappear, owing to their great solubility and the consequent leaching out into the soil. The aleurone layer, then, is not a tissue that stores potassium for the use of the embryo during germination. .
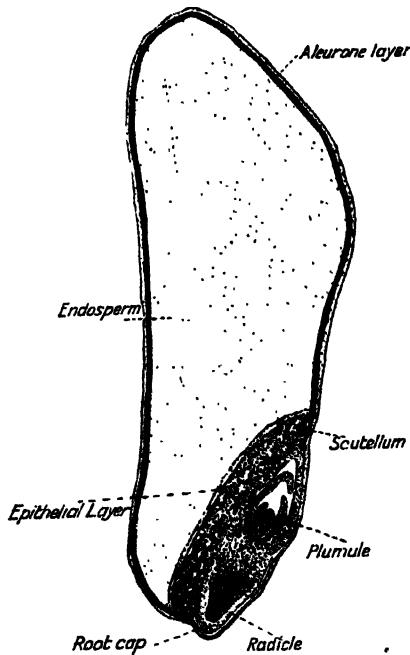
TEXT-FIG. 3. Diagram of distribution of potassium in 'Marquis' wheat after two days' germination, January 11, 1924.

B. *The Embryo.*

The large amount of potassium in the embryo is shown in Text-fig. 3, previously referred to. The scutellum and the root and stem apices contain the greatest amount.

The scutellum usually gives a darker reaction at that end nearest the root, while the epithelial layer contains less potassium and in finer particles. In regard to the very marked contrast of potassium content of the scutellum and endosperm, it may be noted that the cotyledons of the broad bean have also been found rich in potassium, which occurs in the same manner as in the scutellum of the wheat —as a sheet of granules laid down against the cell-wall. It is possible that the foliar origin of the cotyledon may be of some significance in their increase of potassium content over that of the endosperm.

It is of interest that potassium occurs in each tissue in such a characteristic way that the parts of the embryo can be recognized by the form in which the mineral is laid down.

In the scutellum it occurs as a broad black ring of granules against the cell-wall. In the plerome and periblem the potassium takes up a peripheral position in the cell, as shown in Text-fig. 5, while in the dermatogen it is scantier, and occurs as precipitates throughout the cytoplasm, as in

Text-fig. 6. In the root-tip it appears, as it often does in outer tissues, in large localizations.

We cannot at present draw any conclusions concerning the factors governing the particular form in which potassium occurs in the cells of
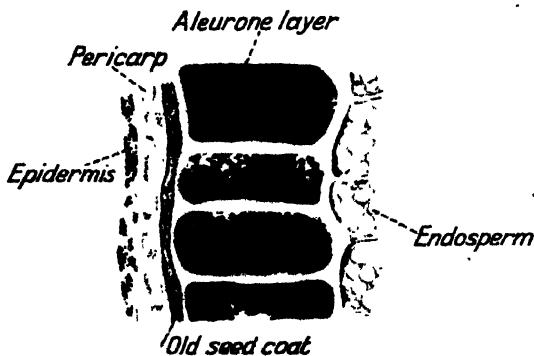
TEXT-FIG. 4. Distribution of potassium in the outer tissues of the wheat grain.
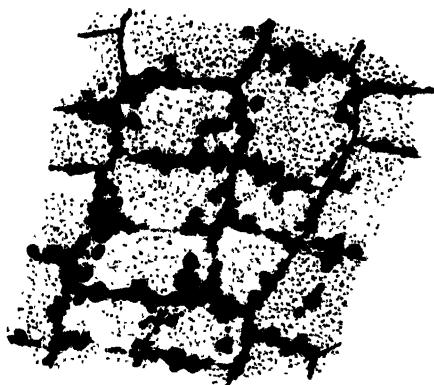
TEXT-FIG. 5. Potassium in cells of plerome of the radicle of 'Marquis' wheat after five days' germination, January 17, 1924.

different tissues. It is not completely determined by the distribution of the cytoplasm, because the distribution of potassium in the outer part of the meristem, shown in Text-fig. 6, differs greatly from its distribution in the inner part, as is shown in Text-fig. 5, and yet the cells of each of these tissues are equally full of cytoplasm.

When the radicle is first differentiated the whole organ is uniformly rich in potassium, but in about a week, when the root has elongated, most of this is translocated to the tip. As it approaches the apex, the con-

ducting strand increases in potassium content, and the root apex, including the meristem, is quite black. In the root-cap the reaction is somewhat fainter.

Priestley (3) states that there is a total absence of potassium in the meristem of willow root-tips, and infers that this may hold for potassium and other electrolytes in all root-tips; the contradictory results here obtained from the study of the roots of spruce and wheat have led me to further investigations of these organs in other plants.

The adventitious roots developing from the twigs of *Salix* were
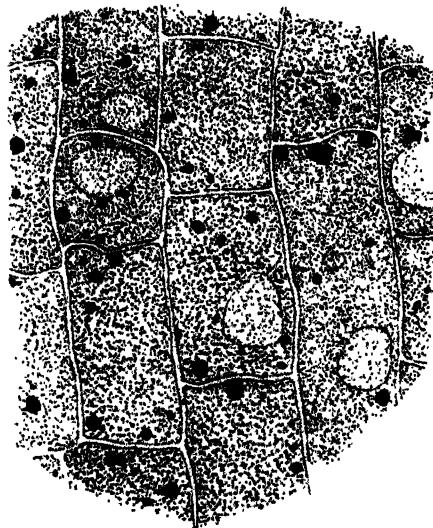
TEXT-FIG. 6. Cells from the dermatogen of the radicle of ' Marquis' wheat after five days' germination, January 17, 1924.

sectioned. After taking great care in the methods, and guarding against any diffusion, it was found that the meristem was black with potassium.

Further, the root-tip of the onion was found to be so extremely rich in potassium that the black reaction in the meristem made it difficult to distinguish individual cells.

Potassium was also plentiful in the meristem of the bean root. In examining this material, it was found that the potassium grains arrange themselves along those walls that are parallel to the long axis of the root more generally than they do along the others.

Again, it has been noticed, especially in the bean root and to a less extent in the wheat root, that potassium is particularly connected with the outgrowth of secondary roots. In the primary root there is always a collection of potassium where the secondary root is given off. In con-

nexion with this, it is of interest that the cortex in the spruce stem is richer in potassium at the region of the branches than it is at the internodes.

Macallum has noted that potassium seems to be connected with the formation of new outgrowths, and that it is particularly plentiful in *Equisetum* spores, where the ' root hairs' (rhizoids) are being developed.

This statement is borne out by the fact that when wheat grains are placed in a potassium chloride solution there is a greater development of secondary roots (and at the same time less elongation) than there is in those grains that have been germinated in distilled water.

6. SUMMARY.

1. Potassium is absent from the wood of the mature root of the spruce during the winter.

2. There is an increase in the potassium content of the vascular system throughout all the organs of the spruce in the spring, and also in the winter, if grown in greenhouse temperature.

3. All meristematic cells are particularly rich in potassium.

4. When the spruce bud elongates there is a translocation of the potassium within the embryonic cone to the next year's meristem and to the embryonic leaves.

5. In the mesophyll of the mature spruce leaf there is an increase in potassium content in the early winter and a decrease in the early spring.

6. In summer the potassium in the mesophyll of the spruce occurs as a network of granules between the chloroplasts. In winter, localizations appear in the proximity of the laked chloroplasts.

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EXPLANATION OF PLATE XII.

Illustrating Mr. Dowding's paper on the Regional and Seasonal Distribution of
Potassium in Plant Tissues.

Fig. 1. Transverse section of the root of *Picea* collected November 26. The relative richness of the different tissues in potassium is shown by depth of shading in Figs. 1, 2, and 3. *xy.* = xylem; *r.d.* = resin duct; *ph.* = phloem : *pcy.* = pericycle; *m.r.* = medullary ray; *ph.* $^{1-3}$ = phellem, phellogen, and phelloderm respectively.

Fig. 2. Transverse section of young stem collected November 15, showing distribution of potassium. *ck.* = cork; *r.d.* = resin duct; *ph.* = phloem; *ctx.* = cortex; *c.* = cambium; *p.* = pith; *xy.* = xylem; *m.r.* = medullary ray; *s.* = sclerenchyma and epidermis.

Fig. 3. Longitudinal section of bud of *Picea* collected November 10, showing distribution of potassium in bud and crown. *m.c.* = meristematic cone; *cr.* = crown; *c.* = cavity; *ctx.* = cortex; *p.* = pith; *xy.* = xylem.

Fig. 4. Mesophyll cells Transverse section of leaf of *Picea* collected November 11 outside. Note the winter condition of cell contents and the connexion between localization of potassium and the 'laked' chlorophyll. *cy.* = cytoplasm; *chl.* = chlorophyll; *o.g.* = oil globule. Potassium shown as dead black.

Fig. 5. Mesophyll cells. Transverse section of leaf of *Picea* collected April 15 outside, showing migration of potassium and of chloroplasts from the nucleus. *n.* = nucleus; *chl.* = chloroplast. Potassium shown as dead black.

Fig. 6. Mesophyll cells. Transverse section leaf of *Picea* collected January 18 from greenhouse material, showing summer condition of chloroplasts and potassium. *chl.* = chloroplast; *cy.* = cytoplasm. Potassium shown as dead black.

DOWDING — DISTRIBUTION OF POTASSIUM.

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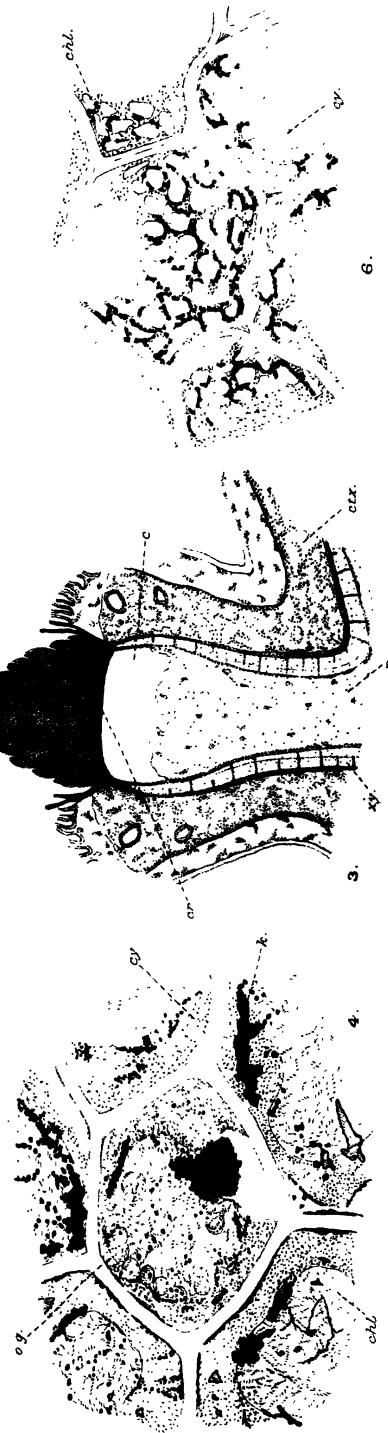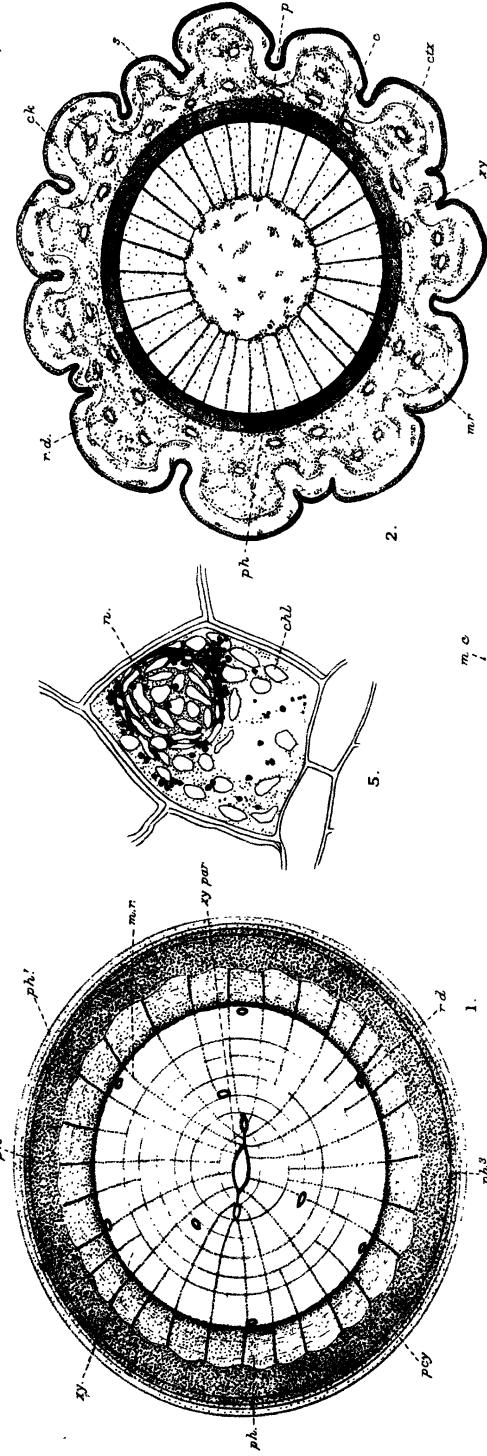
Plant Yield and the Intensity of External Factors-Mitscherlich's 'Wirkungsgesetz'.

BY

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With two Figures in the Text.

THE question as to how much the yield of a crop is increased by increasing the amount of manure at the plants' disposal must have interested man as soon as he made the discovery that the size of his crops could be increased by adding manure. This problem has its physiological as well as its practical and economic aspects.

Many attempts have been made to formulate the relation between the size of the plant yield and the intensity of the external factors affecting the growth of the plant. Liebig was one of the first to make such an attempt. He stated that of all the factors there was one which was ' *in minimum* ', and that the size of the crop was determined by this one factor and increased with increase in this factor until a point was reached at which some other factor was ' *in minimum* '. As to how far this idea that one factor alone determined the size of the yield is connected with the philosophical views of causal relationship then held we are not here concerned. The careful experiments of Mitscherlich and many other workers have shown conclusively that the simple formula of Liebig is not a true expression of the yield-factor relationship.

In attacking such a problem as the one before us there are at least two methods of approach. One is to consider our knowledge of the plant as a working mechanism, and from this knowledge attempt to formulate some law as to the relation we should expect to find between the yield and the intensity of the factors of the environment. For example, something is known of the relation between the rate of some plant processes and the environmental conditions, and yield is an integration of these processes.

When we take stock of such knowledge we find that it is altogether too small and scattered to serve as a basis for the formulation of such laws. The other procedure is to attempt to establish empirically some relationship between yield and external factors. Having established this relationship it may be possible to work backwards and discover its physiological significance, or at least make of it some practical use. From this point of view one is tempted to compare plant physiology with the more exact science of physics. At one stage the knowledge of the structure of the hydrogen atom was not sufficient to forecast the position of the bands in the hydrogen spectrum. The position of the bands was observed for a portion of the spectrum and the position of successive bands formulated mathematically, and this mathematical expression used for forecasting the position of yet other bands. Subsequently a theory of the hydrogen atom was put forward which accounted for the relation previously established.

The part played by the empirical formula in the two cases is, however, somewhat different. In the case of the hydrogen atom the relationship empirically established was used as such until some fundamentally new conception such as Bohr's theory of the atom was formulated. In plant physiology the explanation of the empirically established relation does not await any fundamentally new conception, for explanation here consists of utilizing the conceptions of chemistry and physics ; the difficulty lies rather in the tremendous complication of the living organism as compared with the hydrogen atom : a complication so great that, except to the most sanguine, an explanation of the relation between the size of a plant and the environmental factors seems remote.

· As far as we are aware, no attempt has been made to proceed in the way first mentioned. Mitscherlich, as a matter of fact. has inverted the order rather—he has formulated a law of plant growth on the basis of his law of plant yield.

· The purpose of this paper is to consider (*a*) as to how far the law of plant yield formulated by Mitscherlich—' Wirkungsgesetz '—is an expression of the relation between the yield and the intensity of the environmental factors ; (*b*) the practical use of this expression; and (*c*) its physiological significance.

That the law is considered by Mitscherlich to have some physiological significance is suggested by his frequent reference to it as ' Gesetz der physiologischen Beziehungen '.

Our attention will be concentrated mainly on the limitations of Mitscherlich's law, since Mitscherlich and his supporters have advanced most of the arguments in support of their attitude. Various German workers have criticized the law, but we do not think that the outlook has been that from which the present criticism is made.

Although we do not agree with Mitscherlich in his treatment of his

experimental data, yet we realize the indebtedness of plant physiologists to him for his careful and extensive investigations, ·which give us a qualitative idea of the nature of the relation between yield and manure.

FORMULATION.

The final expression adopted by Mitscherlich (11) is

$$y = A \left(1 - e^{-c_1 x_1}\right)\left(1 - e^{-c_2 x_2}\right) \dots \left(1 - e^{-c_n x_n}\right);$$

y is the yield; A is the maximal yield; x_1, x_2, &c., are the intensities of the factors of the environment; and c_1, c_2, &c., are constants (Wirkungsfaktoren)

FIG. I.
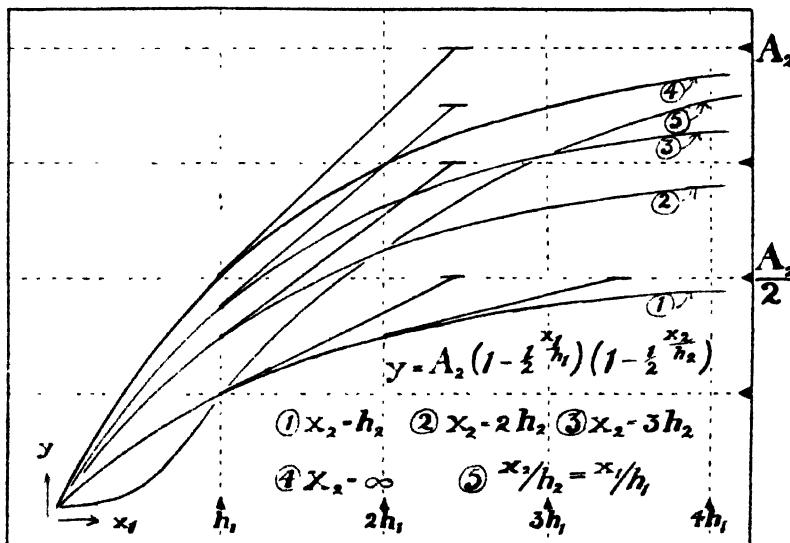
dependent upon the nature of the factor and upon that alone; whilst e is the basis of the natural logarithms.

Considering only one of the factors, x_1, the relation between y and x_1 is given by the expression $y = A_1(1 - e^{-c_1 x_1})$. The value of A_1 is

$$A \left(1 - e^{-c_2 x_2}\right)\left(1 - e^{-c_3 x_3}\right) \dots \left(1 - e^{-c_n x_n}\right),$$

and is the value of y when x_1 is increased to a very large quantity so that further increase has no practical effect on y. When x_1 is zero $e^{-c_1 x_1}$ is equal to unity, and hence y is zero; as x_1 becomes greater and greater, $e^{-c_1 x_1}$ approaches zero, and hence y approaches A_1.

Baule's form of the equation (2) is

$$y = A_1(1 - e^{-0.7\,x_1/h_1}),$$

where h_1 is the value of x_1 when $y = A_1/2 : (e^{-0.7} = \frac{1}{2})$. This may be put in the form
$$y = A_1(1 - \tfrac{1}{2}^{x_1/h_1}).$$
When $x_1 = h_1$, $A_1 - y = A_1/2$; when $x_1 = 2h_1$, $A_1 - y = A_1/4$; when $x_1 = 3h_1$, $A_1 - y = A_1/8$ (see Fig. 1). When $x_1 = \infty$, then $y = A_1$ or $A_2(1 - e^{-c_2 x_2})$ or $A_2(1 - \tfrac{1}{2}^{x_2/h_2})$, where $y = A_2(1 - \tfrac{1}{2}^{x_1/h_1})(1 - \tfrac{1}{2}^{x_2/h_2})$. Thus $A_2 - A_1$ decreases geometrically as x_2 becomes h_2, $2h_2$, $3h_2$, &c. (see Fig. 1). $A_3 - A_2$ is related to x_3 in a similar manner and so on until we come to $A - A_{n-1}$ and x_n.

Another way of expressing the relation between y and any one factor x_1 is

$$\frac{dy}{dx_1} = c_1(A_1 - y).$$

That is, the increase in yield per unit increase in the factor, when this latter is so small as not appreciably to alter y, or the slope of the y, x curve, is proportional to the deficit of the yield at that point from the maximum, $A_1 - y$ (see Fig. 1). It is in terms of increase of yield for increase in manure (perhaps measured in units of money) which the agriculturist thinks.

The form of the equation most favoured by Mitscherlich himself is

$$\log_e (A_1 - y) = \log_e A_1 - c_1 x_1.\,[1]$$

He says (12): 'Ich will mich im folgenden nicht der Bauleschen Formulierung bedienen, da hier der Ausgleich der gefundenen mit den berechneten Werten nicht so exakt durchführbar ist.' In practice it is not the absolute value of x_1 which is measured, but x, the excess of x_1 beyond some value b already present and unknown, and so the expression takes the form

$$\log_e (A_1 - y) = \log_e A_1 - c_1(x + b).$$

When x is zero the value of y is a_1 and

$$\log_e (A_1 - a_1) = \log_e A_1 - c_1 b.$$

Hence $\qquad \log_e (A_1 - y) = \log_e (A_1 - a_1) - c_1 x.$

This is the form used by Mitscherlich in the first place in 1909.

Returning to the original equation (p. 477) it will be seen that according to this expression the yield will be increased by an increase in any of the factors, provided the intensity of the factor is such that $e^{-c_1 x_1}$ is greater than zero. In each case the value of $\dfrac{dy}{dx}$ will be proportional to the corresponding factor c and to the deficit of the yield at that point from the maximum attained when that particular factor is increased indefinitely. Mitscherlich's law is thus definitely different from Liebig's, which postulates that one factor, and one alone, can be *in minimum* in a given set of conditions.

[1] Actually Mitscherlich uses logarithms to the base 10, which are 0·434 times those to the base e :—c_1 is altered proportionately.

If all other factors $x_2 x_3 \ldots x_n$ are increased the value of y cannot rise beyond $A\,(1 - e^{-c_1 x_1})$, and in that sense the yield is *limited* if not *determined* by the value of x_1. This, of course, holds for each factor. A factor might then be said to be *in minimum* when the intensity of all the others is so great that a change in their intensity does not alter y.

Mitscherlich's *present* attitude is that, with certain exceptions,[1] his formula holds for any plant, or portion of plant (corn or straw), and for any time of harvest (8 and 10), the values of c_1, c_2, &c., being unchanged. This amounts to saying that as far as variations in one factor alone are concerned the yield expressed as a fraction of the maximum yield obtained when that factor is very great is the same whatever may be the plant, portion of plant, time of harvest, and other growth conditions (weather, soil, &c.).

For y Mitscherlich uses the harvested quantity. Baule suggests that y is the amount of matter produced, the quantity harvested less that sown. In support of his suggestion Baule advances the following argument (2). He says that an examination of the experimental data of Mitscherlich shows that ' der auf gedüngtem Felde erzielte Ertrag linear mit dem auf ungedüngtem gewonnenen wachst ', but when instead of ' Ernte plus Aussaat' 'Ernte allein ' is used, then ' Die auf gedüngtem Boden erzielte Ernte ist proportional der auf ungedüngtem Boden gewonnenen '. It should be noted that this is in agreement with Mitscherlich's law only when the unmanured soils do not differ in their content of the manure which is being added. If they do differ in this respect the increase of y for a given increase in x is smaller the greater the amount of x already present. Baule assumes that if x were zero then y would be the amount sown.

In the cases where x is mineral salts supplied (and not light or carbon dioxide) the seed usually contains a higher percentage than does the plant produced, and hence it is possible for a seed to produce a plant of normal ash content with a greater dry weight than the seed even when no external supply of salt exists, and thus ' Ernte allein ' would not be zero when x was zero. Baule produces no data on this point. In most cases this quantity is probably so small compared with the experimental error of the values of y that it can be neglected in practice. Of course, as far as the formula

$$\log\,(A - y) = \log\,(A - a) - cx$$

is concerned, this point is not relevant.

Consideration of Experimental Results.

The very extensive experiments carried out by Mitscherlich and his co-workers, as well as those of Hellriegel and Pfeiffer and others too numerous to mention, do show that where only one factor such as potassium is

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[1] Mitscherlich is continually readjusting his position as new facts come to light. Previously he did not maintain that c_1 was the same for all plants.

varied at a time the relation between yield and factor is, generally speaking, of the type expressed by the curves given in Fig. 1, and that the curve for potassium is different for different values of other nutrient (say magnesium) (see Table I).

TABLE I.

Yield of Oats in Sand Cultures.   Potash (x) and Magnesia (z) varied.
(After Mitscherlich (10).)

$\frac{x}{z}$	0·00	0·10	0·25	0·60	1·50
0·00	1·2 ± 0·1	3·1 ± 0·3	4·7 ± 0·4	8·4 ± 0·3	10·4 ± 0·3
0·02		5·2 ± 0·5	8·9 ± 0·3	15·6 ± 0·6	19·8 ± 1·6
	1·2 ± 0·1	4·5 ± 0·4	10·7 ± 1·0	18·5 ± 1·1	25·0 ± 1·3
0·05 {(1)	1·2	6·6	12·6	20·3	25·4
(2)	1·1	5·7	10·7	17·4	24·5
(3)	1·2	4·9	9·9	19·0	25·7
(4)	1·2	4·2	8·8	18·7	25·0
0·12		7·5 ± 1·2	14·7 ± 1·1	21·7 ± 1·5	33·8 ± 1·7
0·25	1·1 ± 0·2	9·5 ± 0·7	17·7 ± 1·3	33·1 ± 1·2	39·8 ± 1·2

Figures in row (1) calculated from log $(A_1 - y) = \log A_1 - 1·07\,(x + 0·02)$

(as used by Mitscherlich)

„   „   (2)   „   „ $y = 34\,\dfrac{x + 0·02}{x + 0·02 + 0·59}$

„   „   (3)   „   „ $y = 1·2 + 38x - 14x^2$

„   „   (4)   „   „ $y = 1·2 + 30x + x^2 - 4·63x^3$

In most cases where manure is added in the form of a salt, two factors are increased simultaneously (urea nitrate and ammonium nitrate are of course an exception). When two factors with different constants are increased simultaneously the value of $\frac{dy}{dx}$ is no longer proportional to the deficit from the maximum.

Suppose $y = A_{1·2}(1 - e^{-c_1 x_1})(1 - e^{-c_2 x_2})$

and $x_2 = kx_1$

$$\frac{dy}{dx_1} = A_{1·2}\{c_1 e^{-c_1 x_1}(1 - e^{-c_2 k x_1}) + kc_2 e^{-c_2 k x_1}(1 - e^{-c_1 x_1})\}$$

$$= A_{1·2}\{c_1 e^{-c_1 x_1} + kc_2 e^{-kc_2 x_1} - (c_1 + kc_2)e^{-(c_1 + kc_2)x_1}\}$$

$$\frac{d^2 y}{dx_1^2} = A_{1·2}\{(c_1 + kc_2)^2 e^{-(c_1 + kc_2)x_1} - c_1^2 e^{-c_1 x_1} - k^2 c_2^2 e^{-kc_2 x_1}\}$$

$$\frac{d^2 y}{dx_1^2} \gtreqless 0 \text{ as } (c_1 + kc_2)^2 e^{-(c_1 + kc_2)x_1} \gtreqless c_1^2 e^{-c_1 x_1} + k^2 c_2^2 e^{-kc_2 x_1}$$

as $(c_1 + kc_2)^2 \gtreqless c_1^2 e^{kc_2 x_1} + k^2 c_2^2 e^{c_1 x_1}$.

When x_1 is zero the right-hand side is equal to $c_1{}^2 + k^2 c_2{}^2$ and hence $\frac{d^2y}{dx_1{}^2} > 0$. Consequently the value of $\frac{dy}{dx}$ rises at first (see Fig. 1, curve 5). The value of x_1 where $\frac{dy}{dx}$ is a maximum depends upon the values of $c_1 c_2$ and k.

If x_2 is so great that $(1 - e^{-c_2 x_2}) = 1$ at the commencement, then the ordinary relation between y and x will hold. This means that if in investigating the effect of a substance such as potassium, a salt such as phosphate or nitrate is added, the conditions should be such that sufficient of the other elements introduced as part of the salt (phosphorus or nitrogen) is already present to render the effect of further increase of these elements negligible.

In most cases (certainly of the later experiments) these precautions are taken by Mitscherlich.

In the case where nitrogen is added in the form of ammonium nitrate or urea nitrate it is justifiable to consider that but one factor is being changed only if the constants are the same for nitrogen in the form of ammonium, urea, and nitrate ions. Mitscherlich states that such is the case (11), but his data are not sufficiently extensive to be convincing. The increments of manure are too great to say that $\frac{dy}{dx}$ does not rise at first as it would in the case of urea nitrate if the constants for the two forms of nitrogen were different, and the amount of nitrogen already present was sufficiently small. More detailed experiments are required to decide this point, particularly since there is evidence that the ammonium and urea nitrogen are not taken in as such by the plant.

This point apart, the question arises as to whether the agreement between the results and those expected on the basis of the ' Wirkungsgesetz ' is of a more than general nature. The question can be resolved into two parts: (*a*) Is the relation between y and x of the form $y = A(1 - e^{-cx})$? i.e. is the value of c a constant when calculated from this equation? (*b*) Is the value of c the same (for the same variable) no matter what is the plant, soil, weather, &c. ? It is possible that c may not be constant and yet the y/A, x relation be the same for all plants, weather, &c.

In support of the validity of his law, Mitscherlich shows that values of y calculated on the basis of his equation using a definite value of c for each external factor agree fairly closely with the values found experimentally, for different kinds of plants under various conditions (10). It seems, however, that more than this is required.

In the first place a fairly close agreement of the calculated and observed values of y is not sufficient proof that the relation between y and x_1 is of the type which Mitscherlich's law postulates. To take a specific case, we will consider some of the results of an experiment carried out with oats to determine the amount of potassium available in a sample of soil (12). The

results will be found in Table II. It can be claimed that these results are in no way exceptional. Taking the results for the total yield of the sand

TABLE II.

Yield of Oats in Mixtures of Sand and Soil with varying Potash.
(After Mitscherlich (12).)

A. Sand, 6 kg. Soil, none.

x	Grain (a). Observed.	Calculated. (1)	(2)	Straw (b). Observed.	Calculated. (1)	(2)	Total (c). Observed.	Calculated. (1)	(2)	(3)
0·00	5·5±0·4	5·7	6·1	10·9±0·4	11·3	10·3	16·3±0·7	17·0	16·1	17·1
0·10	12·3±0·3	12·2	12·7	22·2±0·3	21·2	21·3	34·4±0·6	33·4	33·7	35·5
0·25	16·0±0·3	16·6	16·6	27·3±0·3	27·5	27·9	42·4±0·7	44·1	44·1	43·1
0·60	18·2±0·4	18·8	18·4	29·6±0·6	30·7	30·8	47·8±0·8	49·5	48·7	47·9
1·50	19·4±0·4	19·0	18·5	31·3±0·1	31·0	31·0	50·8±0·4	50·0	50·0	50·5

	B.	**C.**	**D.**
	Sand, 5·5 kg.	5·0 kg.	4·0 kg.
	Soil, 0·5 ,,	1·0 ,,	2·0 ,,

x	Total (c). Observed.	Calculated. (1)	(2)	(3)	Total (c). Observed.	Calculated. (1)	(2)	(3)	Total (c). Observed.	Calculated. (1)	(2)	(3)
0·00	20·8±1·1	24·5	24·2	24·7	36·0±1·8	33·8	36·1	34·7	41·0±2·0	41·2	39·0	40·7
0·10	37·3±1·6	37·7	36·8	37·6	47·2±0·5	45·4	45·0	46·0	43·3±2·0	48·1	44·9	47·4
0·25	50·6±1·1	46·3	46·2	43·7	51·9±1·2	52·9	51·0	51·0	53·0±0·7	52·5	49·9	51·7
0·60	49·3±1·1	50·6	52·1	48·7	54·3±1·8	56·5	53·7	56·6	54·4±1·2	54·8	54·9	55·4
1·50	49·3±4·1	51·0	53·0	50·8	54·2±1·1	57·0	54·0	59·5	55·3±2·0	55·0	55·0	55·0

Calculated values from following equations :

(1) A. *a* $\log(19-y) = \log 19 - 3(x + 0·052)$

(1) A. *b*. $\log(31-y) = \log 31 - 3(x + 0·066)$

(1) A. *c*. $\log(50-y) = \log 50 - 3(x + 0·060)$ as used by Mitscherlich.

(1) B. *c*. $\log(51-y) = \log 51 - 3(x - 0·095)$

(1) C. *c*. $\log(57-y) = \log 57 - 3(x + 0·130)$

(1) D. *c*. $\log(55-y) = \log 55 - 3(x + 0·200)$

In columns (2) A values 18·5, 31·0, 49·0, 53·0, 54·0, and 55·0.

     ,,   *c*   ,,   3·3, 3·3, 3·3, 2·5, 3·0, and 2·0.

     ,,   *b*   ,,   0·53, 0·53, 0·53, 0·106, 0·160, and 0·268.

$$\log(A-y) = \log A - c(x + b)$$

In columns (3) A. *c*, B. *c*, C. *c*, and D. *c*. A values 52·6, 52·6, 61·8, and 60·2.

$$y = A \frac{x+b}{x+b+c}$$

     c   ,,   0·0622

     b   ,,   0·03, 0·055, 0·80, and 0·130.

Values of *b*	per kg. sand.	per kg. soil.
From equations (1)	0·010 grm.	0·080 grm.
,, ,, (2)	0·00883 grm.	0·116 grm.
,, ,, (3)	0·005 grm.	0·055 grm.

cultures, the first point to note is that, as in the majority of cases, the absolute values of *x* (potassium) are not known, but only the successive increments 0·10, 0·15, 0·35, and 0·90 ; and secondly the value of A_1, the maximal yield, is not

known with any more accuracy than are the other values of y. All we can say is that the y, x curve is such that when the abscissae are b, $b+0.1$, $b+0.25$, $b+0.60$ (b an unknown quantity), the ordinates are about 32, 68, 83.5, and 94 per cent. respectively of the value when the abscissa is $b+1.50$. With the data as indefinite as this it cannot be claimed that the relation between y and x is $y = 50(1-e^{-3x})$, as Mitscherlich suggests. As a comparison of the calculated results shows, the results are more nearly in agreement with $y = 50(1-e^{-3.3x})$

$$\text{(making } b = 0.053 \text{ instead of } 0.060\text{),[1]}$$

and, what may appear still more striking, the agreement is yet closer when the values are calculated on the assumption that the relation between y and x is of an altogether different nature, namely $y = A_1 \dfrac{x+b}{x+b+c}$, where $b = 0.03$ and $c_1 = 0.0622$. That this is not an isolated case is shown by a comparison of the observed and calculated results when the sand was mixed with different quantities of soil (the values of b are so chosen that the calculated amount of potassium per kg. of soil and per kg. of sand is the same in all experiments). The application of the same type of formula to the results obtained with mono-calcium phosphate is shown in Table III. Here the formula used is $y = \dfrac{x+0.024}{x+0.024+0.163}$.

TABLE III.

Yield of Oats in Sand Cultures. Phosphorus, as Mono-calcium Phosphate (x) varied. (After Mitscherlich (10).)

	Observed.		Calculated.		
x		(1)	(2)	(3)	(4)
0·00	9·0 ± 0·3	9·0	9·0	9·5	8·5
0·05	19·3 ± 0·5	19·3	19·1	18·8	20·6
0·10	27·2 ± 2·0	27·4	27·2	26·8	28·5
0·20	41·0 ± 0·9	38·7	39·0	39·1	38·2
0·30	43·9 ± 1·1	45·7	46·7	46·9	43·9
0·50	54·9 ± 3·7	52·7	55·0	51·6	50·4
2·00	61·0 ± 2·2	57·0	61·0	61·0	61·0
Sum of squares of deviations (unweighted)		29·4	11·9	24·2	31·7
(Weighted)		2·95	2·05	2·51	2·41

Figures in column (1) calculated from log $(57-y) = 1.6812 - 2.1 x$ (as used by Mitscherlich)

    ,,   ,, (2)   ,,   ,, log $(61-y) = 1.7160 - 1.87 x$

    ,,   ,, (3)   ,,   ,, $y = 9.5 + 20x - 280x^2 + 96.4x^3$

    ,,   ,, (4)   ,,   ,, $y = 66 \dfrac{x+0.024}{x+0.024+0.163}$.

NOTE.—The squares of the deviations are weighted by being divided by

$$\frac{\text{Probable error}}{\text{Mean}} \times 100 \text{ (observed)}.$$

[1] We shall see later that Mitscherlich himself fits to these same results equations with constants which vary according to the occasion (7).

Values calculated on the basis of an equation of the type $y = a + \beta x + \gamma x^2 + \delta x^3$ are also given. As is shown by a comparison of the sum of the squares of the deviations of the calculated from the observed results, the fit of these two equations is not inferior to that of Mitscherlich's equation. Further, an inspection of the table will show that Mitscherlich has not taken those values of A_1 and c_1 which give the best fit. 61 and 1·87 respectively are better values than 57 and 2·1 (the latter chosen to show its constancy under all conditions) as used by Mitscherlich. A variation of 11 per cent. is rather large in the value of c_1.

It seems perfectly clear, then, that with experimental data such as those available the agreement of calculated and observed results has little significance. The crucial data, the values of y for very small values of x, for deciding whether a formula of type $y = A_1 (1 - e^{-c_1 x})$ or of the type $y = A_1 \dfrac{x}{x + c_1}$ is in closer agreement with the results, are missing, and, owing to the complications of substances present in the seed, are likely to be missing for some time. In the case of the results in Table II, according to Mitscherlich's formula $y = 50 (1 - e^{-3x})$, the value for y when $x = 0.02$ is 6·45, whilst according to the formula $y = 50 \dfrac{x}{x + 0.0622} = 1.22$.

The application of a formula of the latter type to a set of results where the smallest value of y is less than 5 per cent. of the maximum observed is shown in Table I.

In the application of his type of formula to results with varying light intensities, instead of having an unknown amount of mineral substance in the sand and seed, Mitscherlich has a minimum light intensity below which the plant does not develop. In the case of oats, for example, this minimum is 0·15 of full sunlight in summer-time, the values of y being calculated by means of the formula

$$\log (110 - y) = 2.0414 - 2.5 (x_1 - 0.15).$$

The flexibility of his formula is thus retained.

Considering the range of the values of c_1 in the formula

$$\log (A_1 - y) = \log A_1 - c_1 (x + b)$$

which, with the uncertainty as to the values of A_1 and b, will give calculated values satisfying Mitscherlich's standards of closeness of fit with the observed, it is not surprising that the results with the same variable x but with different plants and conditions can be fitted with formulae all having the same value for c_1. The constancy of this value of c_1 Mitscherlich advances as evidence of the validity of his law (10). Apart from the points already considered, constancy of c_1 is not sufficient evidence. Let us consider the case of the relation of yield to phosphate for various plants,

years, water-supply, &c., the data for which are collected together by Mitscherlich (10). The values for c, for mono-, di-, and tri-calcium phosphate, and for Thomasmehl are 2·1, 1·4, 0·7, and 0·33. The author gives the equations from which the calculated results are obtained. For evidence as to the accuracy of fit, reference must be made to the original papers (7 &c.). From his formulae the values of A_1, the maximal yield, and a_1, the yield when $x = 0$, can be obtained. These values are given in Table IV.[1]

TABLE IV.

Maximal Yield (A_1) and Yield for no Phosphates added (a_1). Sand Cultures, various Plants and Conditions. (Calculated from Mitscherlich's Equations (10).)

Plant.	Remarks.	A_1.	a_1.	A_1/a_1.	b (as Thomas mehl).
Oats	Original experiments as recorded, 1922	57·0	9·0	6·;	0·226
,,	1913, small water-supply	53·0	6·4	8·3	0·169
,,	1913, greater water-supply	89·0	11·0	8·1	0·174
Pens	1914	46·0	4·7	9·8	0·142
Beans	,,	60·0	28·0	2·1	0·830
Zuckermohrhirse	,,	47·0	2·3	20·5	c·066
Oats	1916, greenhouse	82·6	8·2	10·1	0·138
,,	1916, open	58·0	9·0	6·4	0·222
,,	1916, without CaCO₃	65·0	9·0	7·2	0·196
,,	1917	82.0	12·0	6·8	0·214

Presuming that the sand used did not differ in phosphate content, from experiment to experiment, or from year to year, then if Mitscherlich's law is to apply, not only should c be the same in all cases, but the ratio of y for one value of x to that for another should be constant, and hence among others A_1/a_1 should be the same for all the experiments. An inspection of the values for this ratio shows that such is not the case (see Table IV). The differences cannot all be attributed to sand with different phosphate content being used in different years. For in the same year the ratio is 2·1 for beans and 20·5 for Zuckermohrhirse. (The varying quantities of b, phosphate in the sand, calculated as Thomasmehl, are given in the table.) As to whether differences of this type are due to inclusion in yield of weight of seed sown (in which case the law does not apply as Mitscherlich concludes that it does), or whether they are to be attributed to phosphate contained in the seed, we cannot say.[2] In the case

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[1] It is interesting to note that in the original work on phosphates and oats $A_1 = 67·7$, $a_1 = 9·7$, and $c_1 = 1·5$, 1·0, and 0·5 for the three calcium phosphates (6). Whereas the values are now 57, 9, 2·1, 1·4, and 0·7 respectively. The observed value for a_1 has also changed. This illustrates the flexibility of the formula.

[2] The necessary data are not available, but it does not seem likely that twenty field beans should contain the equivalent of 0·6–0·8 grm. Thomasmehl phosphate.

of oats Mitscherlich usually took thirty-five plants per pot, which would mean about 0·018 grm. of P_2O_5 if each grain contained half a milligramme, which seems to be a liberal allowance. For peas and beans he used twenty plants, and for Zuckermohrhirse he used fifteen plants. If the difference between the different plants is to be attributed to different phosphate content of the seeds, then this should be allowed for in the formulation. The different values for b might be said to indicate a difference in the availability of the phosphorus for the different plants.

Supposing that when the substances in the soil and in the seed have been taken into account, the relation between yield, expressed as a percentage of the maximum, and manure is the same for all plants and conditions of weather, &c., which is one of the points Mitscherlich is attempting to establish, it does not necessarily follow that this relation is of the type formulated by Mitscherlich: it might just as well be of the type $y = A \dfrac{x}{x+c}$, which is as flexible as Mitscherlich's under the conditions.

<div align="center">TABLE V.</div>

Yield of Peas. Supply of $Ca_3(PO_4)_2(x)$ and Grunddüngung (z) varied. Mean yield for $x = 0$ taken as 100. (From Mitscherlich's data (7).)

z x	1	2	4
0·00	100 ± 4	100 ± 4	100 ± 4
0·15	218 ± 9	216 ± 12	194 ± 11
0·40	357 ± 14	334 ± 11	326 ± 7
0·90	478 ± 5	453 ± 12	424 ± 9
4·00	620 ± 19	617 ± 13	655 ± 9

An inspection of Table V shows that in the case of peas it cannot be claimed that the y/A_1-phosphate relation is independent of the other manure added. The data, in no way specially selected, are those given by Mitscherlich for peas in sand culture (7). There are three phosphate series each having a different amount of ‘Grunddüngung’. If the y/A_1 phosphate relationship were independent of other factors, then the yield, in terms of yield when $x = 0$, should be the same for each value of x. It will be seen that even allowing for the probable error there is no definite indication of such being the case.

As for the constancy of c_1 for different times of harvest there are no data on this point as far as we are aware, and if such did exist the above criticisms would apply with the same force as in the above cases.

The application of the formula to different portions of the plant will be considered in a future section.

We will summarize this section with the assistance of Fig. 2. Mitscherlich's experiments supply us with points $p'-p''''$. We know the position of the ox axis approximately, since the ordinates are known approximately,[1] but we do not know that of the oy axis. Mitscherlich finds the latter by drawing a curve somewhere about the points, and produces it until it cuts the ox axis. He has no grounds for assuming his curve to be correct. It is not the one of nearest fit and cuts the ox axis at different points for different plants, different portions of plants, and different weather conditions. We have no criterion as to where it should cut ox.

Of those ardent supporters of Mitscherlich who may claim that,
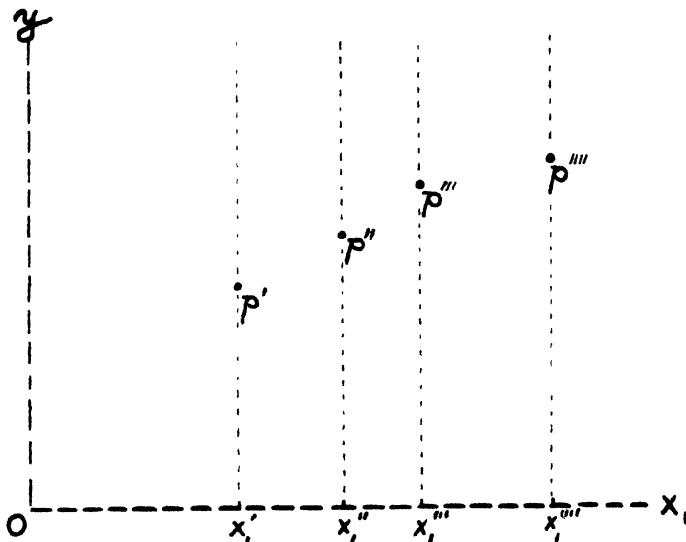
FIG 2.

although the fit of the observed results to the theoretical curves is not very good, Mitscherlich has succeeded in showing that there is a fundamental constant for each manure, we would ask, What then is the value of this constant? In the case of Thomasmehl. is it 0·85, or 0·31 as Mitscherlich calculated in 1916, or 0·33 as he calculated from the same data in 1922, or yet some other figure which gives a closer fit of calculated and observed results, or that calculated on the basis of some other type of equation?

Exceptions.

There is a number of cases in which the law fails to apply in a very obvious manner. For example, in the case of varying quantities of potash the value for c is as great as 2·2 when the nitrogen is applied in the form

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[1] Only approximately, since the ordinates are y/A_1, both v and A_1 not being fixed definitely.

of sodium nitrate,[1] whereas when the nitrogen is in the form of ammonium nitrate $c = 1 \cdot 07$, and when ammonium sulphate the value is reduced yet lower. Mitscherlich explains this state of affairs as due to reactions in the soil ; in the first case the sodium from the sodium nitrate liberates potassium and so increases its availability, whereas in the last case the soil becomes acid, and hence there is a depressant action. Explanations of this kind may be correct, but it seems to us that similar interactions take place to a greater or less extent in all cases.

The number of exceptional cases is increasing as time goes on. Mitscherlich now (12) admits that the 'constant' for carbon dioxide is dependent upon the light intensity, the value increasing with the intensity of the light since. ' Wird durch grössere Lichtzufuhr . . . die Assimilationstätigkeit der Pflanze gehoben, so wird dadurch das Diffusionsgefälle der Kohlensäure von den intermizellaren Hohlräumen zu der Atmosphäre grösser, und damit der Zutritt weiterer Kohlensäuremengen zur Assimilation beschleunigt.' Accepting the validity of the argument, there seems no reason why it should be confined to the case of carbon dioxide. In the same paper Mitscherlich admits that the constant for water can be increased by accelerating the solution of the nutrient substances, or by bringing them into the immediate neighbourhood of the plant.

Practical Use of Mitscherlich's Equation.

Mitscherlich has attempted to use his equation in conjunction with the results of pot-culture experiments to determine (a) the amount of a substance in a sample of soil (12), and (b) the effect of the addition of artificial manure to field plots (8). We will first consider the practical aspects of the question.

In Table II are recorded the results of experiments with oats on mixtures of sand and a limestone soil, using varying quantities of potassium sulphate. He has assumed that the value of c for potash under these conditions is 3, and that it is the same for all the soil mixtures. The value of 3 is higher than usual on account of the presence of sodium nitrate in the Grunddüngung. In view of this, the only justification for assuming c to be the same for all sand-soil mixtures is the agreement of the observed and calculated results— a justification which carries no great weight.

In applying the equation :

$$\log (A - y) = \log A - 3 (b + x),$$

the value of x, the amount of added potash, alone is known. There is the restriction that b must be the sum of the amounts of potash in the sand and

[1] In one paper (11) he uses a value as high as 3.

in the soil, but this still leaves considerable latitude in fixing the value of b (of course having fixed the values of b for any two of the experiments it is then fixed for the other two).[1] With this adaptability of A and b it is not surprising that values are found so that the values of y calculated on their basis give with the observed an agreement which Mitscherlich considers satisfactory.

Mitscherlich's values for b are not the values which give the best fit. As an example we will consider his total yield in the unmixed sand cultures. Assuming with Mitscherlich that $A = 50$ and $b = 0.06$, then for each value of y with its corresponding x the value of c can be calculated by means of the formula

$$c = \log \frac{50}{50-y} \Big/ (x + 0.06).$$

The different values of c obtained are then weighted in each case according to the size of the square of the coefficient of variation[2] of the respective mean values of y. The mean of these weighted values is of the order 2.6 and not 3.0. Consequently it may be concluded that with $A = 50$ $c = 3$ the value of b, which gives the best fit, is not 0.06. Mitscherlich advances no evidence that 50 is the value of A, which gives the best fit. It seems clear that the guiding principle of assigning values to A was that c should be 3 and b such that the amount of potash in sand and soil deduced from any pair of equations should agree with that from any other pair. Not having the bias as to the immutability of c in the presence of a limestone soil, but accepting the restriction as to b, we have applied other equations with the result that the potash in the sand and soil becomes 0.0088 grm. and 0.116 grm. per kg. respectively, as compared with Mitscherlich's figures of 0.01 and 0.08. It cannot be claimed that the calculated values of y show a worse agreement with the observed than those calculated by Mitscherlich.[3]

We will turn for a moment to some theoretical considerations. Suppose that the relation between y/A_1, and x_1, the total amount of potash in the soil, is independent of the percentage of soil in the sand-soil mixture (there is no *a priori* reason for so supposing, especially since there is evidence as to the dependence of the relation upon a form of nitrogen supply), then, no matter what the form of the equation expressing this relation, it would be possible, from experiments in which the effect on y of further supplies of potash had been determined, to discover the amount of potash already present.

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[1] There is no reason apart from the validity of Mitscherlich's law why with c constant for all experiments b should be limited by these restrictions.

[2] Proportional to probable error divided by the mean.

[3] We have not bothered to restrict ourselves to choosing values of A and c which give the nearest fit. We merely wished to show that other values of A and c would fit as well as those of Mitscherlich.

For example, if we assume that the relation between y/A_1 and x_1 is of the form expressed by the equation $y = A_1 \dfrac{x_1}{x_1 + c_1}$, where c_1 for potash is 0·0622, then the agreement of the calculated and observed values of y (see Table II) indicates that the amount of potash already present is 0·005 grm. per kg. of sand, 0·055 grm. per kg. of soil. As our previous considerations have shown, there is no reason for preferring an equation of the type suggested by Mitscherlich to this other type (not that we hold any brief for the latter), and consequently there is no reason for preferring his values for potash in the soil to those indicated by the other formula, particularly as neither values have any significance in themselves, but, given the experimental data, are implicit in the y/A_1, x_1 relation assumed. It may again be pointed out that there is no evidence for the constancy of c_1 under all conditions in either type of equation.

The values of b in the equations assumed by Mitscherlich are smaller in the case of grain than in the case of the straw—the equations for the sand culture alone are quoted. As Mitscherlich says, ' Das dürfte zunächst vielleicht verwundern ', but, he continues, ' ist dadurch erklärlich, dass sich erst das Stroh bilden muss, und die Pflanze somit bestimmte Nährstoffmengen aus dem Boden aufnimmt, welche nach dem für die Kornerträge nicht mehr im Boden zur Verfügung stehen können '.

That in this particular case. for Mitscherlich's standard of agreement of calculated and observed results, it is not necessary to assume b different for grain and straw is shown by the application of equations in which b is the same for both (admittedly we have chosen a different value for c from that used by Mitscherlich).

Apart from this point there seems no reason why the straw should take some of the potash already present in the sand, leaving less for the grain, and not do the same with the potash added. If it did so the value for c_1 should be different for grain and straw—smaller for the former. Assuming the general validity of the ' Wirkungsgesetz ', either the different values of b for grain and straw or the identity of c_1 are fictitious. Mitscherlich's is certainly a very ingenious explanation of the complicated problem of the effect of manure on the balance of the reproductive and vegetative portions of a plant, for if c_1 is the same for both and it is certain that the ratio of ' grain ' to ' straw ' does shift with change of some manures, then must b be different for ' grain ' and ' straw '.[1]

In the paper from which the above experimental results have been taken (12) the data are confined to one plant. As we have seen on page 486, the formulae applied by Mitscherlich imply a variation of b with the plant. As we then pointed out, this variation of b may indicate

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[1] In as far as the calculated values of corn and straw, using $c_1 = 3\cdot3$ in above case, agree with those observed, the ratio of corn to straw does not shift.

a difference in the availability of mineral nutrient (phosphates in this case) for different plants, or a difference in the seed content of that nutrient factor. In either case b has not the significance attached to it by Mitscherlich.[1] On the other hand, it may be an indication that Mitscherlich's Wirkungsgesetz does not hold.

We now come to the other practical use of Mitscherlich's law—the forecasting of the effect of manure on field plots. This is really, in the main, the same problem as the last. Knowing the y/A_1, x_1 relation we can, having established A_1, and y for some known value of $x_1 - b$, deduce b, the amount of x_1 already present, and the effect of an increase of x_1. As in the previous case, this holds whatever the nature of the relation, and the fact that we can do so is no evidence for or against the Wirkungsgesetz, as its author seems to imagine (11).

All the previous evidence considered is based upon pot cultures. The question arises as to how the data of these are to be used in field cultures. In the first place Mitscherlich has shown that the manure must be converted from grammes per pot into weight per area. From pot cultures it is possible to determine the effect of addition of manure to a given volume of the soil. Suppose that the addition of p grm. of potash per pot results in a reduction of $A_1 - y$ by 50 per cent. If p grm. per pot is equal to q grm. per unit area of soil, we cannot say that an application of this amount to a field culture will result in a reduction of $A_1 - y$ by 50 per cent. In the pot the plant has a limited depth of soil at its disposal, in the field this quantity is not fixed. In as far as this means an alteration in the intensity of all other factors it should affect A_1 and y in the field proportionately as compared with A_1 and y in the pot (assuming Mitscherlich's law to hold), but it also means an alteration in the variable, potash. Mitscherlich has drawn attention to these points in his paper. The value of b deduced from field experiments is usually greater than in pot experiments, thus suggesting a greater depth of soil available in the former: but it should be noted that most of the field experiments were carried out with potatoes, whilst the pot experiments were performed with oats. As far as b is smaller for the pot cultures it means that the effect of a given increase in manure will be less in the field. In one case where oats were used in both cultures the value for b was smaller in the field, but larger for beans and potatoes (Table XVI, loc. cit.). This seems a peculiar state of affairs—if Mitscherlich's law applies.

Application to Standraum.

In his paper on the relation between yield and spacing (9) Mitscherlich says, ' Bei der Verarbeitung unserer Resultate, habe ich auch für die Abhängigkeit des Pflanzenertrages einer bestimmten Fläche y von der

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[1] See the title of paper (12).

Dichte des Pflanzenbestandes x in erster Annäherung unser Gesetz der physiologischen Beziehungen in Ansatz gebracht':

$$y = E\,(1 - e^{-k_2 x}).'$$

He also uses $w = A\,(1 - e^{-k_1 u})$, where w is the weight of an individual plant. According to his summary this also is used 'in erster Annäherung': x is the number of plants on an area q.

$$u = \frac{q}{x} \text{ and } w = \frac{y}{x}.$$

If both equations are to apply, certain conditions must be fulfilled.

$$y = wx = Ax\,(1 - e^{-k_1 q / x})$$

when $x = \infty$, $y = Ak_1 q$, also E ∴ $E = Ak_1 q$

$$w = \frac{E}{x}\,(1 - e^{-k_2 x}) = \frac{Eu}{q}\,(1 - e^{-k_2 q / u})$$

when $u = \infty$, $w = Ek_2$, also A ∴ $E = \frac{A}{k_2}$.

Hence $k_1 k_2 = \frac{1}{q}$.

$k_1 k_2 = \frac{1}{q}$ and $E = \frac{A}{k_2}$ are the conditions that the two equations shall be in agreement at $x = \infty$ or $u = 0$ and $u = \infty$ or $x = 0$. If these conditions are fulfilled, it does not mean that for all values of x and u the two equations are equivalent. Mitscherlich seems to be under this impression, for he says (p. 345, loc. cit.), 'Können wir die Richtigkeit der Gleichungen (5) und (8) $(E = Ak_1 q = A/k_2)$ am Schlusse an unserem Beobachtungsmaterial erhärten, so sind wir berechtigt, in beiden Fällen, ganz gleich ob wir die Beobachtungen auf die Einheit der Pflanze oder auf die der Standraumfläche beziehen, in jedem Falle das Gesetz der physiologischen Beziehungen in erster Annäherung in Ansatz zu bringen'.

A numerical example will perhaps elucidate this point.

According to the first equation

$$y = E\,(1 - e^{-k_2 x}).$$

According to the second, putting $w = \frac{y}{x}$, $A = Ek_2$, $u = \frac{q}{x}$, and $k_1 = \frac{1}{k_2 q}$,

$$y = Ek_2 x\,(1 - e^{-1/k_2 x}).$$

For successive values of $k_2 x$, 0, 1, 2, 4, 5, and ∞, the values of y/E by the first equation are 0, 0·63, 0·86, 0·98, 0·993, and 1, by the second 0, 0·63 0·79, 0·88, 0·907, and 1. Consequently if $k_1 k_2 = \frac{1}{q}$ and $E = A/k_2$ the equations cannot both fit the results unless the standard of agreement is very low.

Mitscherlich fits equations to his results for yield per pot and yield per plant, and finds that $E = k_1 Aq$ and $A = Ek_2$, and hence concludes that either equation is applicable. Apart from the fact that his procedure was probably, one might say certainly, reversed: he obtained values of A, E, k_1, and k_2 which fitted the above conditions, and used these values in his equations: his equations are not those which fit the results best.[1] For example, he fits an equation, $\log (182 - y) = 2.2601 - 0.182 x$, to his results for White Mustard (his Table II). Assuming his value for $E = 182$, the values of k_2 were calculated for 1, 3, 6, and 12 plants, using $\log (182 - y) = 2.2601 - k_2 x$. These values were weighted according to the square of the reciprocal of the percentage probable error. The weighted mean of k_2 was found to be 0.149, and not 0.182. Of course, Mitscherlich gives no evidence for using 182 for the value of E. These points in conjunction with a comparison of his calculated and observed results show that his claims have little foundation.[2]

Whatever Mitscherlich may mean by saying that the yield of a single plant w is related to the area of soil per plant u, according to the expression $w = A (1 - e^{-k_1 u})$ as a first approximation, it seems clear that this has no relation to his Wirkungsgesetz, since, supposing that an increase in u means a proportionate increase in the values of the food available in the soil (this may be approximately the case for smaller values of u), it means an increase in n factors, not one factor. Only if, and as far as, all factors but one were present in a very great intensity could a relationship of the Mitscherlich single factor type be expected to hold, granted Mitscherlich's law is true for such factors.

The data are not sufficiently accurate or extensive to say definitely whether $\dfrac{dw}{du}$ falls off from the beginning as it does for the single factor relation.[3] It seems to us that the experiments show that the relation between yield per area and number of seeds, and that between yield per plant and 'Standraum', are of a non-linear type, and in a very general way of the type expressed by Mitscherlich's single factor equation—but only in a very general way.

Standraum in Mitscherlich's Pot Cultures.

This raises the question of the effect of spacing in Mitscherlich's pot cultures, on the results of which he bases his generalizations embodied in

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[1] In fact throughout Mitscherlich's work, in all the cases we have tested this criticism holds.

[2] His Table II shows observed results 44 ± 4.6, 51 ± 2.3, 47 ± 3.8, and 47 ± 1.6, and calculated 37, 47, 51, and 52.

[3] If $\dfrac{dw}{du}$ rises at first as in the multiple factor curve, then $\dfrac{dy}{dx}$ in the yield, density of sowing curve will not fall away continuously.

his law. As far as we are aware, no investigations have been made as to the interaction of spacing and manure in pot cultures, and consequently one cannot say that the value for c_1 for an external factor is independent of the number of plants in a pot.

In many of Mitscherlich's experiments he used thirty-five plants per pot in the case of oats and barley, and twenty plants in the case of field beans and peas. The numbers are probably nearer the region where the yield per pot is independent of the number of plants than the region where the yield approaches one of proportionality to the number of plants—that is, when all the nutrient factors of the soil are quite high.

Let us suppose that the yield per pot is independent of the number of plants per pot—there is experimental evidence for such a state of affairs (14) —and further that Mitscherlich's equation

$$y = A (1 - e^{-c_1 x_1}) (1 - e^{-c_2 x_2}) \ldots \ldots (1 - e^{-c_n x_n})$$

applies to the yield of a single plant. If n be the number of plants and the yield per pot Y

$$Y = nA (1 - e^{-c_1 x_1}) \ldots \ldots (1 - e^{-c_n x_n}).$$

If by increasing n (say doubling) Y is not changed, then, since A is a constant, the product of the bracketed terms must be correspondingly decreased (halved). Suppose for the sake of argument that the number of factors is twenty, and that the terms $c_1 x_1$, $c_2 x_2$, &c., are all equal. If the product of the bracketed terms is halved, then each term is reduced $\frac{1}{\sqrt[20]{2}} = 96.5$ per cent of its previous value. This means that $c_1 x_1$, &c., are of the order of 6.7 ($e^{-6.7}$ is about 0.00122 and $e^{-3.35}$ about 0.035). Now consider the case where x_1 is one-tenth of the value in the above case and x_2, &c., unchanged. If the number of plants is now doubled, the value of $(1 - e^{-c_1 x_1})$ is changed from

$$(1 - e^{-0.67}) = 0.488 \text{ to } (1 - e^{-0.335}) = 0.286,$$

and hence Y is changed from

$$nA (0.488) (0.9988)^{19} = 0.476 \, An$$

to

$$2nA (0.286) (0.9650)^{19} = 0.297 \, An,$$

whereas in the previous case Y is changed from

$$nA (0.9988)^{20} = 0.973 \, An$$

to

$$2nA (0.9650)^{20} = 0.978 \, An.$$

Consequently, if n plants per pot were used, the ratio of the yield when $c_1 x_1 = 0.67$ to that when $c_1 x_1 = 6.7$ would be 0.49, and when $2n$ plants were used the maximal yield would be the same as before, but the ratio would be 0.30. Therefore the value of the constant, the parameter, of the yield-factor curve is a function of the number of plants. If c_1 is a constant for

all plants when the yield of only one plant is considered, it will be the same for all plants when the yield per pot is considered when, and only when, a certain definite number of plants are grown in each pot. This number will be a specific or varietal index. We have no grounds for assuming that Mitscherlich has hit off the right numbers when he uses thirty-five oat plants, twenty bean plants, and fifteen millet plants.

In the above consideration, which leads us to the not unreasonable conclusion that the effect of an increase in manure is greater the more crowded are the plants, we have assumed that doubling the number of plants halves the intensity of all the factors. In the case of light this is not quite true, unless the whole area of the pot is covered with plants in the first case. That we do not really attach any great importance is, however, evident from our attitude towards the premise as to the relation between yield and intensity of external factors. What we wish to emphasize is that Mitscherlich has no practical evidence for the assumption that the yield (per pot) factor relationship is independent of the number of plants per pot; on the other hand, if his law does hold for the yield (per plant) factor relationship, there seems to be an indication that the relationship for yield per pot is a function of the number of plants.

There is yet another point in this connexion. If we suppose that in the case of pot cultures the root systems ramify all over the medium, then the whole of the mineral content may be considered as available. If, then, when one of the nutrients is decreased, the root system becomes larger, the amount of the available salts will be unchanged; in field crops, however, the root system may penetrate to yet greater depths, and hence when one factor is decreased others may be increased. Consequently, Mitscherlich's law should not hold for both pot cultures and field cultures, as the author claims. Variation of area of surface available is most probably connected with variation in depth of soil invaded by the root system. We shall consider at a later stage a few of the many more complications of a somewhat similar nature.

Problems such as those considered in this section are in need of experimental investigation.

Another Possible Source of Error in Technique.

The procedure in the pot cultures is to sow more seeds than the final number of plants required, and after successful germination to eliminate the extra plants. Let us consider the case where the supply of phosphates, for example, is varied. It is most probable that the percentage of phosphorus in the seed will vary from one individual to another. If the supply of phosphorus per plant is that in the seed plus a fraction (according to the number of plants) of that supplied, then when the amount supplied is great the percentage difference in the total supply per plant will be small, whereas

when the amount added is small the percentage difference will tend to be greater. If these assumptions are valid we should expect, provided no other factor is introduced, that the variability of the yield would be greater for small manurial supply than for large. Mitscherlich's means and probable errors are unfortunately for pots and not for plants. If this difference appears in the early stages of growth, we should expect a larger percentage of relatively poor germinations[1] under the conditions of low phosphate supply. This certainly is so in many cases. That being the case, it seems very likely that the tendency in eliminating extra plants would be to eliminate those weakly established, particularly if this difficulty had not been realized, and, as far as we are aware, it is not mentioned by Mitscherlich. In the case of high phosphate supply the distribution of success in germination would tend to be normal, and hence, in eliminating extra plants, leaving as uniform a sample as possible, as Mitscherlich states he does, the tendency would be to remove the plants of extreme size and leave those from the average seed. With the low phosphate supply the mode would tend to be towards the bigger side of the mean, and hence the plants left would tend to be those from seed with higher phosphorus content. Consequently the yield would tend to be higher than that from average seed, and more so the smaller the value of x (the manure added), and if the results as observed obey Mitscherlich's law, the results for uniform samples of seed throughout all cultures would not.

Physiological Considerations.

In attempting to form a picture as to the effect of an environmental factor on the size of plant, one possible course is to attempt to consider the problem from the point of view of effect of the factor on the rate of growth, and further, as a commencement, to simplify the problem by substituting a colony of unicellular organisms for a higher organism.

As far as we know, the mature size of a bacterium is not affected by the concentration of any one nutrient substance. The fact that over a certain range the relative growth-rate is proportional to the concentration of nutrient suggests that the size of the individual is unaffected (13).

If y is the size of a colony of unicellular organisms such as yeast or bacteria,[2] then for an appreciable time the rate of increase is $\dfrac{dy}{dt} = ky$, the size at any moment being given by the equation $y = y_0 c^{kt}$ or

$$\frac{\log_e y - \log_e y_0}{t} = k,$$

where y_0 is the size at $t = 0$ and e the base of the natural logarithms. The relative growth-rate, $k = \dfrac{dy}{dt} \Big/ y$, after remaining constant for some time,

[1] By germination is meant successful establishment of young plant.
[2] Size of colony = number of individuals.

falls off, in some cases due to exhaustion of food, in others due to some internal changes in the individuals connected possibly with the products of metabolism.

When we turn our attention to the higher plants, apart from minor fluctuations, the relative growth-rate falls off throughout the life-cycle. As to what is the changing set of conditions to which this is to be attributed we do not know. One aspect of it may be called increasing differentiation—an increasing amount of the plant becomes non-productive. The way in which the relative growth-rate changes with time is most probably very complicated. Simple formulae have been suggested.

The formula put forward by Robertson (15) and others is

$$\frac{dy}{dt} \Big/ y = k\,(A - y),$$

A being the final size.

Mitscherlich (5) suggests $y = A\,(1 - e^{-ct})^n$; the relative growth-rate then becomes

$$\frac{dy}{dt} \Big/ y = nc\,\frac{e^{-ct}}{1 - e^{-ct}}.$$

Baule (3) suggests $y = A\,(1 - e^{-ct^n})$.

The equations have, it seems to us, as much significance as Mitscherlich's Wirkungsgesetz.

In the case of the colony of cells, the final size will be proportional to the initial amount of nutrient if the composition of the cells is unaffected and growth proceeds so that the relative growth-rate is proportional to the concentration. This proportionality will not hold when t is not very great. If the cell content of the variable nutrient increases with the external concentration of this nutrient, then, instead of the final size of the colony being proportional to the initial amount of this variable nutrient, the size will be relatively greater for the smaller amounts. If the growth of the colony is inhibited by a certain concentration of products of metabolism, then inhibition will take place at an earlier stage (size relative to possible size) in the colony with high concentration of variable nutrient—this factor will disturb the proportionality in the same way as the previously mentioned factor.

Returning to the higher plant, we undoubtedly have a non-proportionality of yield to supply, of the above-mentioned type. As in the falling off of the relative growth-rate, this non-proportionality is due to a complicated and changing set of conditions. Fluctuation in composition of plant with variation of nutrient may be one aspect, inhibition of growth may be another. If the change in relative growth-rate with time was independent of the amount of variable nutrient the relative growth-rate would have to be some complicated function of concentration of nutrient in order that y (the final yield) should be proportional to the initial amount of

nutrient, as a consideration of the Robertson or Mitscherlich formula would show.

In attempting to follow the growth of the plant in time, we are faced with the complication that in some cases, where all the nutrients are in solution at first, the concentration is falling with time and at different rates and different relative rates with the different concentrations, whereas in those cases where the form in which the nutrient is absorbed by the plant is produced by solution (relatively insoluble substances) or bacterial activity (probably in the case of substances containing nitrogen in other forms than nitrate) the concentration will remain constant for a certain time.[1] Consequently, if in two cases the ratio of x_1 (as used by Mitscherlich) is R, the ratio of concentration will not be R throughout the experiment. In the early stages R (for the concentrations of available x_1) may be unity, and hence one would expect that there would be no difference in y for some time. Such is the case for the cotton plant for various degrees of ' Standraum ' (1), and an investigation of the early stages for different amounts of manure is a problem that requires investigation.

The onset of the reproductive phase in a higher plant, which is closely associated with changes in the relative growth-rate, since growing-points change over from leaf production to flower production, is affected by the mineral nutrients available and has far-reaching effects on the further salt uptake of the plant. The effect of varying amounts of nitrogen, for example, on the relation of reproductive parts to vegetation is a well-known case. Mitscherlich's explanation of the shift of this relation has already received notice.

Investigations of Hoagland, (5) and others have demonstrated the fact that increased concentrations of nutrient solutions have their most marked effects when applied in the early stages of growth—some of Mitscherlich's experiments also illustrate this point, hence, possibly, his procedure of giving the whole supply at the outset. Burd (4) has shown that during the reproductive stage in some cereals the plant actually loses part of its mineral content. This is probably associated with the movement of salts from the stems and leaves to the forming grain.

So far we have considered the plant portion of the plant-environment complex. The variation of one factor of the environment will undoubtedly alter the whole environment part of the complex. As we have seen, Mitscherlich admits that some of these cases are obviously beyond the rule of the ' Wirkungsgesetz '. It seems to us, however, that these are only the extreme cases of what always happens when one factor is varied. Not only is there the physical condition of the soil, but there is the algal, bacterial, and protozoan population, all interconnected and affecting the plants' food-

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[1] In Mitscherlich's experiments the manure was usually all added at the beginning, but not so in the case of water-supply.

supply. All these are inevitably affected by change in amount of nitrate, phosphate, water, &c.

Enough has been said to make clear that any attempt to formulate a law of plant yield based on our present knowledge is impossible.

Possible Yield-factor Relations.

In approaching the subject from Mitscherlich's direction we are not limited to his narrow path. From our general knowledge of plants it is unlikely that when the conditions are made more and more favourable the size of a plant can go on increasing without limit. There are various possible relations : the law of diminishing returns may hold throughout the whole range ; the yield may increase proportionally with increased quantity of manure up to a certain point and then no further, as Liebig suggested ; the return per outlay may increase with increased outlay up to a certain point and then no further increase in the return will result ; or we may have various combinations of these relations, but beyond a maximum return we cannot go.

In the case of the plant and variation in one factor of the environment the law of diminishing returns seems to apply, but one cannot be certain about the state of affairs for very small intensities, since the seed is always provided with a small quantity of nutrient factor.

Now, the Mitscherlich one-factor equation is not the only equation which approaches a maximum value with ever-decreasing return per outlay as outlay is increased. The number of equations is legion and no one has advanced any physiological grounds for accepting this particular form.

Mitscherlich's equation is the same as the equation for a monomolecular chemical reaction—$y = A_1 (1 - e^{-k_1 t})$ where a substance in concentration A_1 at time $t = 0$ and $A_1 - y$ at time t is converted irreversibly into another substance whose concentration at $t = 0$ is zero and t is y, and k_1 the velocity constant—with the substitution of the amount of manure (&c.) available for time. This analogy, however, does not seem very helpful.

In the earlier portions of this paper another type of equation was used for fitting to the experimental results. This equation,

$$y = A_1 \frac{x_1}{x_1 + c_1},$$

approaches a maximum, A_1, as x_1 becomes very great and $\frac{dy}{dx_1} = A_1 \frac{c_1}{(x_1 + c_1)^2}$ decreases as x_1 increases, and hence satisfies the two conditions.

This equation has at least the advantage of having a mental picture behind it. We may perhaps indicate in terms of physics and chemistry what this picture would be.

If we imagine that the seeds sown in a pot contain some substance

capable of producing, when combined with the suitable elements, an amount of plant substance A, that this substance combines with the various elements according to the law of mass action, the combination being reversible, then when the intensity of the non-variable factors is $x_2, x_3 \ldots x_n$, the maximal possible amount of plant substance is A_1, and for the variable factor x_1 we shall have $c_1 y = (x_1 - y)(A_1 - y)$, where y is the amount of plant substance for x_1 and c_1 the dissociation constant of y. (It is assumed for simplicity that one molecule of x_1 goes to form one of y, and that c_1 is independent of x_2, x_3, &c.)

If the amount of x_1 combined is negligible compared with the amount available,

$$y = A_1 \frac{x_1}{x_1 + c_1}.$$

Such a picture is capable of a considerable amount of elaboration, such as, allowing for cases in which x combined is not a negligible fraction of that available, for a variation in c_1 with changes of x_2, x_3, &c. In many cases the corresponding equation would still fulfil the conditions of $y = A$ when $x_1 = \infty$ and $\frac{dy}{dx}$ falling with x_1 rising, and from what we have seen earlier we could make the results, calculated and observed, fit sufficiently well without undue strain on the equation and the imagination.

Recently Sapěhin (16) has suggested 'dass die Adsorption die Hauptrolle bei der Nährstoffaufnahme durch Pflanzenwurzeln spielt', and then, after quoting evidence in support of this statement, he says : 'Dies alles führt zu der Annahme, dass die Ertragsgleichung' ($V = V_1 p^\lambda$, where V = yield, p = quantity of electrolyte at plants' disposal, and $1 > \lambda > 0$) · als von der Adsorptionsisotherme stammend zu betrachten sei.'

He then proceeds to compare calculated and observed values with the usual deductions. After what has already been said it will suffice if we point out that Sapěhin's equation allows for plants or yields of unlimited size, and that his variable is the amount of manure added at the outset, and not the concentration.

So far we have been assuming that the maximum yield was approached asymptotically as the intensity of the external factor was increased.

We know that, in practice, as the amount of nutrient (a salt for example) in the soil is increased, a point is reached where further increase of nutrient is accompanied by a decrease in the yield.

We may say that this is to be attributed to a lack of balance of salts in the soil solution, or to the increased osmotic pressure of the solution, or in the case of light to a depressant effect on the growth-rate of the leaves or what not. But whatever the explanation it seems certain that, in many cases at least, this deleterious effect does not suddenly make its appearance when a certain state of affairs is reached. It is conceivable that in some cases it does. In the case where the depressant effect at super-optimal

concentrations is due to a disturbance of the physiological balance part of the depressant effect of infra-optimal concentrations will also be due to lack of balance. If these super-optimal conditions are to be ruled out as exceptional, and Mitscherlich makes no allowance for them, then the values of the yield in the included range require some modification. The other alternative is that the law should apply to the whole range.

In the case of the latter alternative the equation would be of a different type from that put forward by Mitscherlich: $y = a + bx + cx^2$, where c is negative, a and b positive, for instance, would do. In any case, however, it would, in the present state of knowledge, be a purely empirical equation.

SUMMARY.

Mitscherlich has formulated a law expressing the relation (with certain exceptions) between the yield and the intensity of the external factors governing growth. For a given intensity of external factor (amount of manure added at the outset, fraction of daylight above minimum falling on plants, &c.), the yield expressed as a fraction of the maximal yield (the yield when the intensity of external factors is great) is the same for all plants and conditions, and is equal to $(1 - e^{-c_1 x_1})$.

In the case of manures x_1, the intensity of the factor, cannot be measured directly, since there is a certain quantity in the seed and in the soil. With the yield (y) and maximal yield (A_1) fixed only approximately and this unknown quantity of manure already present (b), the above expression is so flexible that results calculated from formulae with various values of A_1, c_1, and b agree fairly closely with the results observed. Moreover, the results can be fitted with equations of a quite different type, e.g. $\dfrac{y}{A_1} = \dfrac{x_1}{x_1 + c_1}$ or $y = ax_1 + bx_1^2 + cx_1^3$, &c.

Since b, and hence x_1, cannot be determined except by means of an application of some equation to experimental results, it cannot be claimed that y/A_1 is independent of all factors except x_1.

To determine the amount of available manure in a sample of soil (together with that in the seed) it is sufficient to know the curve of y/A_1 against nx_1: it is not necessary that the equation expressing this relation should have any special form.

Pot cultures are of no use in forecasting the effect of a supply of manure on field cultures, since the volume of soil in the latter and the quantity of each of the nutrients contained are unknown.

If the law does hold for the yield of a single plant, the value of c_1, in the case of the yield per pot, is probably a function of the number of plants in the pot, this function probably varying with species.

Errors probably arise in the process of eliminating extra plants after germination.

A consideration of our knowledge as to the development of a plant and as to the effect of variation of nutrient supply at different stages suggests that the agreement of figures, calculated from any simple expression such as that of Mitscherlich, with results from pot cultures in which the nutrient changes with time in an unknown fashion, can have little physiological significance.

The writer is indebted to Dr. F. F. Blackman for reading the manuscript and making suggestions.

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The Development and Distribution of Chlorophyll in Roots of Flowering Plants grown in the Light.

BY

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With nine Figures in the Text.

I. INTRODUCTION.

THE effect of light on roots has been studied chiefly in relation to growth—comparisons have been made between the rate of growth in light, in darkness, and in sudden changes from one to the other. Leitch, in her experiments on the influence of temperature on the rate of growth in *Pisum*, carried out as a control some experiments on the influence of light and came to the conclusion that, at least in the case of the experiments which she was performing, light exerts no influence on the rate of growth (9).

Other observers have studied the heliotropic response of roots. On the subject of the development and distribution of chlorophyll, however, few references could be found. Dr. Arber mentions the occurrence of chlorophyll in the roots of water plants grown under natural conditions. 'Aquatic roots', she states, 'often exercise another function, which is more remote from those generally assumed in the case of terrestrial plants— namely, that of assimilation; their colour is sometimes quite conspicuously green' (1). Noack, in his work on the relation between plastids and chondriosomes, used *Elodea* because—'Die häufig auftretenden grünen Adventivwurzeln erweisen sich als ganz vorzüglich geeignet zur Lebenduntersuchung der Plastiden im Vegetationspunkt' (12). The present account deals with the development and distribution of chlorophyll in roots under various experimental conditions.

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[1] Thesis approved for the Degree of Master of Science in the University of London.

II. EXPERIMENTAL METHODS AND RESULTS.

A. *Distribution of chlorophyll in illuminated roots of different species.*

1. *Method employed.*

Seedlings of sixteen different species were used in this experiment. The seeds were germinated and the seedlings allowed to grow in darkness for about two days (except for a few of the species which were 'earth-grown') and then planted out with their roots passing through holes in a sheet of cork, the cork being supported by four thin glass rods on the rim of a beaker, which was filled with water.

The beakers containing the various seedlings were then arranged around a 200 c.p. lamp, in a deep white sink. The electric bulb was suspended in a large glass jar, which was weighted down with lead, and the sink was filled with water until the water was almost on a level with the rims of the beakers. This arrangement served both to prevent the temperature from becoming too high and to render the illumination as intense and uniform as possible.

The roots were kept well aerated by means of a stream of water passing into each beaker from the tap by way of a piece of rubber tubing, from which the water dripped into a small funnel fixed through the centre of the sheet of cork carrying the seedlings. An overflow from the sink was arranged to prevent the water level becoming too high and so swamping the seedlings.

By such means it was possible to keep the seedlings growing in a healthy condition and free from algae for six weeks, if required.

Controls were grown in the dark.

Experimental Results.

After about a fortnight's growth in the light, transverse sections were made of the roots of all the species and thirteen out of the sixteen species were found to have developed chlorophyll. The distribution of the chlorophyll in the root, as given below, was constant for any given species.

(a) *In stele only—none in cortex.*

Vicia Faba, L.⎱
Rumex sp. ⎰ In various parenchymatous cells throughout the stele.

Acer Pseudoplatanus, L. In two rows of cells above the protoxylem groups.

Aesculus Hippocastanum, L. In the rays and in outer parenchymatous cells of pericycle.

(b) *In stele and in cortex.*

Pisum sativum, L. (7 var.). Very distinct green zone in centre of root. Chloroplasts in the parenchymatous cells of all the stele tissues. Also in cells of endodermis and inner cortex (Fig. 1).

The different varieties all showed the same distribution, the only difference observable being one of intensity.

Vicia sativa, L. Chiefly in the cells of the inner and middle cortex, but also in the outer parenchymatous cells of the stele.

Zea Mays, L. In pith : in cortex sharply limited to one layer outside endodermis (Fig. 2).

FIG. 2. Transverse section of root of *Zea Mays*, L.. grown in light, showing distribution of chloroplasts. Fresh material.
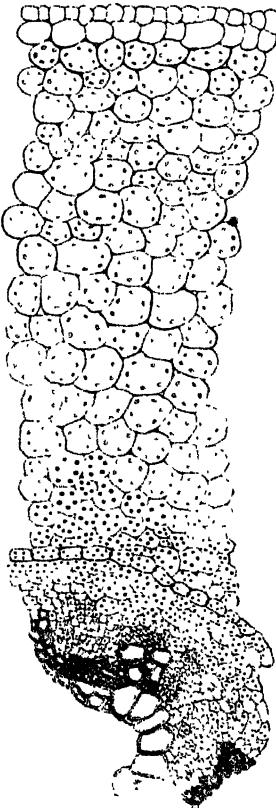
FIG. 1. Transverse section of root of *Pisum sativum*, L., grown in light, showing distribution of plastids. Plastids shown diagrammatically: colourless plastids in outline. Chloroplasts in black. Fresh material.
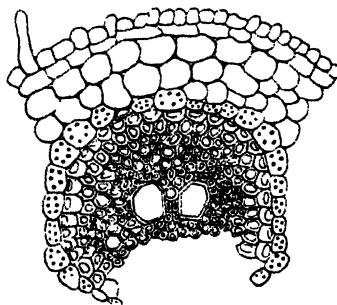
FIG. 3. Transverse section of root of *Triticum vulgare*. Vill , grown in light, showing distribution of chloroplasts. Fresh material.

(c) *In cortex but not within the stele.*

Triticum vulgare, Vill. Sharply limited to one layer outside endodermis (cf. *Zea Mays*, L. In *Triticum* there is no pith, the primary xylem reaching right to the centre) (Fig. 3).

Ranunculus Ficaria, L. (fibrous roots). Sharply limited to one layer outside endodermis (cf. tuberous roots below).

Hordeum vulgare, L.   Limited to about three layers outside endodermis.

Scilla nutans, Sm.   In middle cortex of thick contractile root (Fig. 4).

Helianthus annuus, L.   Throughout cortex.

Bellis perennis, L.   Throughout cortex (Fig. 5.)
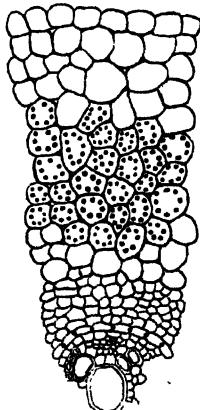
FIG. 4. Transverse section of contractile root of *Scilla nutans*, Sm., grown in light, showing distribution of chloroplasts. Fresh material.
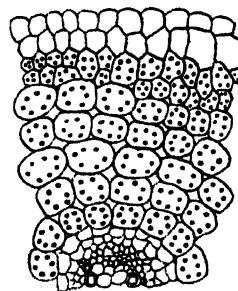
FIG. 5. Transverse section of root of *Bellis perennis*. L., grown in light, showing distribution of chloroplasts. Fresh material.

(d) *No chlorophyll developed.*

Ranunculus Ficaria, L. (tuberous roots).

Ricinus communis, L. '

Fagopyrum esculentum, Moench.

Allium Cepa, L.   (Conditions of experiment unfavourable to growth of this species.)

Longitudinal sections showed that in all species except *Triticum* and *Hordeum* the chlorophyll was developed throughout the main roots to within 1–2 cm. of the apex, the greenness decreasing in intensity from the base to the apex.   In *Triticum* and *Hordeum*, however, the chlorophyll was developed only in the first 1·5–2 cm. of the base of some of the roots and not at all in others.

No chlorophyll was developed in the controls grown in the dark.

B.   *Effect of various conditions on the development of chlorophyll in the roots of Pisum sativum.*

1.   *Roots illuminated, shoots in darkness.*

It was thought possible that the development of chlorophyll in the roots might be conditioned by the formation of substances during assimilation,

Therefore, with this in mind, the general method described above (A 1) was employed, but with the shoots in the dark, a small light-proof shade being placed on the cork sheet.

The seedlings so grown showed only a very slight development of chlorophyll compared with those grown for the same length of time with both shoots and roots in the light.

2. *Shoots illuminated, roots in darkness.*

In order that the roots should be in darkness, the beaker was placed inside a blackened tin, while the shoots were exposed to the light of the 200 c.p. lamp.

No chlorophyll was developed in these roots except just at the base, complete exclusion of light from which was difficult.

3. *Delayed illumination of seedlings.*

Other seedlings were grown for a fortnight in the dark and then transferred to the light, both root and shoot being illuminated.

These seedlings even after a month's illumination showed scarcely any development of chlorophyll in their roots, although the leaves became green and the stem faintly so.

This experiment then, like Experiment B 1 above, suggests that the formation of chlorophyll in the roots is favoured by conditions advantageous to assimilation in the shoot.

4. *Removal of Cotyledons and Epicotyl.*

The cotyledons and epicotyl were removed from some of the vigorous seedlings growing in the light.

The effect of this operation was that the main roots of these seedlings ceased to grow and became, after a few days, green to their tips. Later, new lateral rootlets were formed in which the chlorophyll did not extend to the tip.

In this case, the production of chlorophyll in the root tip was probably due to the maturing of the root-tip tissue.

C. *The effect of culture solutions on the development of chlorophyll in roots.*

1. *Method employed.*

The same general method as described above was employed, but the roots of the seedlings were placed in:

        (i) 0·2 per cent. sugar solution.

        (ii) 0·2 per cent. potassium nitrate solution.

Controls were grown in tap water.

In this case no means of aeration of the roots was devised, but the solutions were changed daily. The species used in this experiment were: *Pisum sativum*, L., *Triticum vulgare*, Vill., *Hordeum vulgare*, L., *Zea Mays*, L., *Vicia Faba*, L.

After some weeks' growth in these two solutions the roots of the seedlings all showed a similar distribution of chlorophyll to those grown in tap water.

D. *Roots grown in damp air.*

It was thought possible that the development of chlorophyll in illuminated roots might be dependent on the degree of aeration, which must always be relatively poor in roots growing immersed in a solution. Some seedlings of *Pisum sativum*, L., were therefore grown in specimen tubes with a thin strip of damp filter-paper dipping into water down one side of the tube.

After a period of illumination the distribution of chlorophyll in the roots was investigated and found to be the same as in those grown entirely immersed in water, so that the poor aeration of roots growing in solution did not appear to be a relevant factor.

E. *Examination of material fixed for plastids and chondriosomes.*

In the course of the work the possibility suggested itself that the cultures might provide material which would throw some light on the vexed question of the relation between plastids and chondriosomes. It has been suggested by some workers, e. g. Guillermond (7), that plastids have their origin in the chondriosomes·which normally seem to occur in embryonic cells—i. e. that the chondriosomes of mature cells are potential plastids which have not developed. By others, e. g. Mottier (10), it is held that in embryonic cells there are two distinct classes of bodies—plastid initials (primordia)—which develop into the plastids of the mature cell, and chondriosomes which do not give rise to plastids but which maintain their characteristics in the mature cells, the function of which is still a matter of conjecture. It was thought possible that as the tissues of the roots became green on exposure to light, some evidence of the transition from chondriosomes to plastids might be obtained.

A detailed study of the distribution of plastids and chondriosomes was therefore made in *Pisum* roots, both of those grown in the light and in the dark. The fixatives used were:

(1) Twenty vol. of 10 per cent. solution of ordinary commercial formalin together with eighty vol. of 3 per cent. solution of potassium bichromate as recommended by N. H. Cowdry for fixing chondriosomes (4).

(2) Weak chromo-acetic acid solution.

The former of these should preserve the plastids and chondriosomes; the latter, the plastids only.   The stains used were:

(1) Iron-alum-haematoxylin.

(2) Acid fuchsin and picric acid.

FIG. 6.   Cell from outer cortex of root of *Pisum sativum*, I.., grown in light, showing nucleus, plastids (some with starch inclusions), and chondriosomes. Drawn with the camera lucida from a prepared slide, fixed with formalin-potassium bichromate, stained iron-alum-haematoxylin.
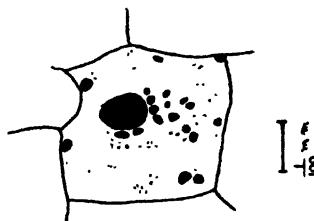
FIG. 7.   Cell from 'green region' of root of *Pisum sativum*, I.., grown in light, showing nucleus, chloroplasts, and chondriosomes. Drawn with the camera lucida from a prepared slide.
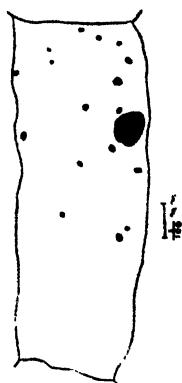
FIG. 8.   Cell from region corresponding to that of Fig. 6, of root of *Pisum sativum* L., grown in dark, showing nucleus, plastids, and chondriosomes.   Drawn with the camera lucida from a prepared slide.
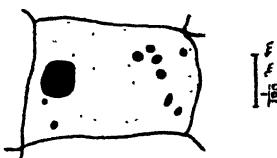
FIG. 9.   Cell from region corresponding to that of Fig. 7, of root of *Pisum sativum*, L., grown in dark, showing nucleus, plastids, and chondriosomes. Drawn with the camera lucida from a prepared slide.

A study of the fixed and stained material soon showed that the hope that the material would be specially favourable for observing transition stages between chondriosomes and plastids was not fulfilled, for well-developed plastids were present in all cells of the cortex as well as in the green zone (Fig. 1).   If anything, the colourless plastids in the cortex (Fig. 6) were larger than those of the green zone (Fig. 7).   In short, the illumina-

tion of the roots caused the development of chlorophyll, but apparently did not affect the distribution of the plastids. In the controls grown in the dark, a similar distribution of plastids was found. In this case, however, all the plastids appeared to be smaller (Figs. 8, 9).

In the material fixed with bichromate-formalin mixture, chondriosomes were observable in the form of small granular or rod-shaped bodies occurring together in the same cell (Fig. 6) much as described and figured by Noack (12). Chondriosomes appeared to be present together with plastids in all the parenchymatous cells of the mature root tissue (Figs. 6, 7, 8, 9). In a number of cells the chondriosomes seemed especially numerous in the neighbourhood of the nucleus. Guillermond noticed a migration of the chondriosomes to and from the nucleus in living material (6).

In the embryonic tissues of the root there appeared to be present some bodies of intermediate size as well as the undoubted plastids and chondriosomes.

No distinct chondriosomes could be made out in corresponding material fixed in weak chromo-acetic acid solution, although the plastids were preserved.

DISCUSSION OF RESULTS.

These experiments seem to show that most, it not all, roots have the potential capability of developing chlorophyll. In which cells of the root the chlorophyll is developed seems to depend on the species. In this respect the work of D. G. Scott is of interest (14). She has recorded the difference in distribution of chlorophyll in the young shoots of woody plants. She examined twenty-four species and found that the distribution varied considerably, and that in these shoots chlorophyll was often developed in situations where light could not penetrate to any great extent, e. g. in the medullary rays and in the pith. Two of the species examined by her were used in this experiment, namely, *Aesculus Hippocastanum*, L.— ' Chlorophyll developed in cortex, scantily in the medullary rays, and in the medullary cells bordering on the protoxylem'; and *Acer Pseudoplatanus*, L.— ' Chlorophyll developed in the cortex, medullary rays (especially in the very broad ones), and in the medullary cells bordering on the protoxylem '. The shoots of the species used in the present experiment showed, when cut, a similar distribution of chlorophyll to that of the roots, except that there was in all cases, in addition, a development of chlorophyll in at least the outer layers of the cortex.

The reason for the development of chlorophyll in some cells and not in others is not at all clear. It certainly cannot be explained as due to the presence of undersized or deformed plastids in the colourless cells, as has been found to be the case with many variegated plants (13). In *Pisum*, at

any rate. the plastids in the outer cortex appear to be particularly large and well formed (Fig. 6).

The fact that the plastids, whether green or colourless, are larger in the light grown parts is of interest. This may be due to the direct effect of light or to better nutrition, owing to assimilation or to some other indirect effect of light. Experiments on etiolated shoots fit in with such explanations as these. That the amount of nutritive material available may influence the development of chlorophyll is suggested by the work of Coupin (3) upon the development of chlorophyll in etiolated seedlings. Coupin found that when etiolated seedlings were exposed to diffuse light, the time of exposure to light required for the production of chlorophyll was least in those regions, e. g. cotyledons, in which nutritive material was most abundant. It appears unlikely, however, that the development of chlorophyll only in certain sharply delimited regions of the root, as occurs in some species, can be due to such a cause. On the other hand, it is possible that rather than light exercising a positive effect, encouraging the development of larger plastids and chlorophyll in some cells, the effect is a negative one due to darkness, i. e. in the dark substances may be produced which have a depressant effect.

The development of chlorophyll in the root seems to be closely correlated with the development of chlorophyll in the stem, for in the case of those seedlings whose shoots were covered up while their roots were exposed to the light, after being in the dark for fourteen days, there was only a very slight development of chlorophyll in both stem and root even after a month's subsequent exposure to the light, although the leaves developed the normal amount.

The presence of both plastids and chondriosomes in all cells throughout the tissues, even in the root tips, is noteworthy. It was, however, almost impossible to decide whether there were any structures of intermediate size present or not. In the older cells the structures were clearly divisible into these two classes, but in the root tip this could not be said to be the case, as there were certainly some granules which might have been said to have been either large chondriosomes or else plastids which had just undergone division. However, in tracing the cell contents from the root tip to the mature tissues, there did not appear to be any striking increase in number of plastids and corresponding decrease in number of chondriosomes, as would be expected if Guillermond's view of the origin of plastids from chondriosomes were correct (7).

In the course of writing this account, my attention has been called to a notice of a paper by A. Siebert (16) dealing with the greening capacity of roots. Unfortunately I have been unable to obtain a copy of this paper.

SUMMARY OF RESULTS.

1. It was found that chlorophyll was developed in the roots of thirteen out of sixteen species grown with their roots exposed to the light of a 200-candle-power electric lamp. Moreover, it was found that the distribution of chlorophyll in the roots was constant for any given species, but varied very considerably from species to species; in some cases the green zone was sharply delimited (e. g. in *Triticum vulgare*, Vill.) and formed a very striking feature in transverse sections.

2. The experiments on the effect of various conditions on the development of chlorophyll in the roots of *Pisum sativum* suggested that the formation of chlorophyll in the roots was favoured by conditions advantageous to assimilation in the shoot.

3. The effect of culture solutions on the development of chlorophyll in the roots was a negative one, the development and distribution of the chlorophyll being similar to that of roots grown in tap water.

4. Seedlings grown in damp air showed the same distribution of chlorophyll as those grown in water, so that the poor aeration of roots growing in solution did not appear to be a relevant factor in the distribution of chlorophyll.

5. In an examination of fixed material, well-developed plastids were found to be present in all the cells of the cortex as well as in the green zone, i. e. the effect of illumination of the root was to cause the development of chlorophyll in some cells, and not to affect the distribution of the plastids.

6. Chondriosomes appeared to be present together with the plastids in all the cells of both the light and dark grown roots.

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Plant Electricity.

I. Photo-electric Currents associated with the Activity of Chlorophyll in Plants.

BY

J. C. WALLER, M.A., F.L.S.

With six Figures in the Text.

CONTENTS.

INTRODUCTION.

WHEN light falls upon a plant tissue, there ensues under certain conditions a production of electricity by that tissue. This photo-electric current or photo-electric response [1] is of interest not merely from the point of view of pure chemistry or physics, but on account of a distinct significance which it appears to possess in reference to plant metabolism : for leaves give this response by virtue of their living state, and not through any properties which they may possess in common with non-living bodies. If this were not so, the response would be of little biological interest. But its physiological nature is clearly indicated by the fact that it is increased, modified, or abolished by just those conditions which arc known to influence vital activities.

In the following paper is summarized the evidence, so far available, that it is the activity of *chloroplasts* which plays a predominant part in the production of electricity by plant tissues under the influence of light. It must be mentioned at the outset that organs such as pulvini, which show specialized phototropic or photonastic reactions, are not dealt with.

Apart from its intrinsic interest, it seems possible that the electrical response may in the future give us some hints concerning the nature of photochemical reactions in the plant ; for the electrical response must itself be a direct outcome of these reactions.

HISTORICAL SUMMARY AND COMMENTS.

The literature of photo-electric currents in plants is comparatively small in volume. Haake (1) appears to have been the first to observe altera-tions of potential in plants consequent upon changes of illumination. His thesis was that electric currents in tissues are in the main brought about by chemical changes, and his conclusions may best be summarized by a quota-tion from his paper (p. 481) translated as follows :

' I. It is established beyond doubt that metabolic changes of a distinct kind are concerned in the production of electric currents in plants. In particular it has been shown that oxygen respiration in the first place, and then again carbonic acid assimilation, are prominently concerned in it.

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[1] The term ' photo-electric effect ' is generally used by physicists in reference to the ejection of electrons by various substances under the influence of light, especially ultra-violet light. Leaves may also be caused to exhibit this phenomenon (McClelland, J. A., and Fitzgerald, R., Proc. Roy. Irish Acad., vol. xxxiii, sect. A (1916), pp. 1–8, ' Photo-electric Discharge from Leaves '), and chlorophyll films, &c. (Dixon, H. H., and Poole, H. H., Sci. Proc. Roy. Dublin Soc., vol. xvi (1920), pp. 63–77, ' Photosynthesis and the Electronic Theory ').

It has not yet been shown whether this emission of electrons is of a physiological nature, but it evidently belongs to a different order of phenomena from the photo-electric currents dealt with in the present paper.

' II. Movement of water possibly has a share in the causative conditions of the electric currents, but certainly its influence is but slight.'

By means of a Lippmann's capillary electrometer, Haake detected changes of potential consequent upon illumination of the leaves of various plants, consequent also upon cessation of illumination. Petals (with one doubtful exception) and other plant organs devoid of chlorophyll gave negative results. Haake therefore concluded that the electric changes are associated with carbon assimilation. He attempted to confirm his conclusion by testing whether the response could be influenced by keeping a leaf in air deprived of carbon dioxide ; in this he failed, and attributed his failure to the impossibility of depriving the internal atmosphere in a tissue of its carbon dioxide. He also tried the effect of augmenting the supply of carbon dioxide, but without definite result. Both of these effects were later demonstrated by A. D. Waller, using more advanced methods than those of Haake (see below).

Klein (2) published a number of observations showing that fluctuations of potential are caused by illuminating and darkening the leaves of various plants. He used the same method as Haake, but his work was done from a different point of view ; not with the idea of establishing a connexion between photo-electric and photo-chemical changes, but with the idea of discovering some relation between the direction of the photo-electric current and the direction of the current (' normal current ') which was flowing in the leaf when first connected to the electrodes at the beginning of each experiment. He was not able to establish any relationship, nor does the attempt appear likely to open up any promising line of inquiry when we consider the uncertainty of the normal current (see below under the heading ' The " initial current " ').

In 1900 the subject was reopened by A. D. Waller (3–6), who employed a Thomson mirror-galvanometer. It was through the study of the photo-electric currents of the retina that he was led to test whether a green leaf would exhibit similar phenomena. An account of his first experiment, performed on March 30, 1900, with an Iris leaf, is given in ' Hitchcock Lectures ', Chapter IV (9), and in the original paper (3). The essential point in his method was that, instead of exposing the entire surface of the leaf to light, only a portion was exposed while the rest remained in darkness. By this means definite differences of chemical activity were brought about between illuminated and obscured portions of the leaf, resulting in clearly defined differences of electrical potential between the two portions.

That Haake and Klein were able to detect photo-electric changes in leaves must have been due to the fact that they placed one electrode upon a vein, the other electrode upon the mesophyll. Light falling upon the lamina would naturally arouse a greater chemical activity in the photo-

synthetic tissue than in the cells of the vein, which latter are less rich in chloroplasts than are the cells of the mesophyll. Thus differences of potential would be brought about between the two regions. But such variations of electrical state are not likely to be so marked and regular as those found by means of Waller's method.

By means also of photographic records of the electrical changes and their calibration in fractions of a volt, Waller set the subject upon a quantitative basis and obtained results which appear to the writer to open up a promising line for investigation in plant physiology.

The chief points to which attention was drawn by Waller (3–6) in the four brief papers of 1900 must now be mentioned. He demonstrated abolition of the photo-electric response of a leaf by boiling or by the action of anaesthetics, thus proving the physiological nature of the response. He studied the effect of different temperatures upon the response, its variation with the time of day, with the age of the leaf, and with its starch-content. He discovered that the leaves of different kinds of plants show characteristic differences in the direction of the photo-electric current both during and after illumination. He also noted the absence of response in certain leaves and concluded that ' the absence of distinct response in petals indicates that chloroplasts are essential to the reaction '. He demonstrated an augmentation of response in consequence of slight increase in the carbon dioxide-content of the air surrounding a leaf; an abolition by excess of carbon dioxide; and a modification in carbon dioxide-free air which was followed by recovery in normal air. He measured the latent period of the response and mentioned the occurrence of fatigue (due to repeated illuminations) and of recovery. He also compared the effects of bright sunlight, diffuse daylight, electrical arc light, and of red, blue, and green lights.

The *results* of the above researches will be referred to by the writer at appropriate points in the course of his own investigations.

Querton (7), who worked in conjunction with Waller, published a number of photographic records which are of special interest in conjunction with the numerical data published by Waller.

A different view from that of previous workers is taken by Bose (8), who regards light as a stimulus similar in its action to any other stimulus—mechanical, chemical, thermal, electrical. He attributes the photo-electric response of plant tissues to their ' excitability', and not to photosynthetic or other specific photochemical reactions. An observation, however, upon etiolated celery (8, p. 396) is interesting as showing an actual case of electric response to light in the absence of chlorophyll (see below under ' Etiolation'). Further theories and observations by Bose, including those more recently published (10, 13), will be considered in a future paper.

A possible interpretation of photo-electric currents of leaves in terms

of ionic changes was announced by A. D. Waller in the Hitchcock Lectures for 1909 (9).

<div align="center">METHOD.</div>

(a) *Apparatus.*

The apparatus was similar in essentials to that used by A. D. Waller. A shield of black paper was laid upon the upper surface of the leaf, so that about half of the leaf remained shaded and half exposed (see Fig. 1).

To lead off the current, a pair of du Bois-Raymond's unpolarizable electrodes,[1] made with U-tubes about 4 mm. in diameter, were placed in contact with the under surface of the leaf, one on the shaded side, the other on the exposed. The electrodes were placed upon areas of mesophyll situated as symmetrically as possible in reference to the veins. The kaolin was made into a paste with sodium chloride solution 0·6 grm. per cent. If required, the surface of the kaolin was moistened with tap water. The strength of the salt solution chosen is arbitrary, but the leaves appeared not to be injured by it. In later experiments the kaolin was made up with tap water. This does not appear to affect the results.

Fig. 1. The plant chamber. The leaf is partially shaded by a shield of black paper. The electrodes, held by adjustable stands, make contact with the lower surface of the leaf. Illumination is by an electric lamp whose heat rays are cut off by passage through a glass dish of water. Light is admitted or cut off by means of a shutter (not shown) between the lamp and the water bath.

In A. D. Waller's experiments the electrodes were in contact with the upper surface of the leaf: this arrangement has the possible advantage that the current is led off from the region of specialized photosynthetic tissue. In all the following experiments (except in the case of very thick leaves) the

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[1] It may be useful here to state how these are made. A smooth rod of zinc dips into a saturated solution of zinc sulphate which in turn communicates with a plug of kaolin made into a paste with saline or tap water. To amalgamate the zinc rod, moisten it with amalgamating fluid and then rub it with filter-paper or a white cloth until the rod is found to be quite clean of superfluous mercury. Amalgamating fluid is made as follows : Dissolve 3 c.c. of mercury in 50 c.c. of $KNO_3 + 150$ c.c. of HCl, and then make up to 1 litre by adding 10 per cent. HCl (after A. D. Waller, Exercises in Practical Physiology, Part III, Longmans, London, 1897).

electrodes were in contact with the lower surface; in this way no shadow was cast upon the exposed part of the leaf by its electrode, but on the other hand a certain number of stomata may be occluded.

It is necessary of course to be assured that the electrodes themselves are not the seat of photo-electric currents. That this is not so under my conditions of experiment is indicated by records of various petals and of boiled leaves, in which there was absence of response. In this case, of course, the metallic parts of the electrodes would be more or less equally illuminated. A more exacting test was therefore made by placing the clay pads of the two electrodes in contact, shielding the metallic part of one electrode while exposing the other electrode to illumination. With the water bath in position and the lamp at 21 cm. distance, no photo-electric current could be detected.

Thus all photo-electric currents observed must originate in the plant tissue, and not in the electrodes.

The plant or detached leaf was placed in a dark box with an aperture in the top, through which light could be admitted or cut off by the movement of a shutter. A glass vessel containing water about 5 cm. deep cut off heat rays.

In the case of detached leaves and petals, unless otherwise stated, the cut end of the stalk was kept in water during the experiment. With some petals, however, e. g. Begonia and Cactus, this was not practicable. When experimenting with cut leaves, &c., it was found convenient to use a smaller box than that shown in Fig. 1.

The source of light was a 100-watt gas-filled electric lamp which by vertical movement could be adjusted at approximate distances from the leaf surface of 21, 30, 42·5, 60, 85, or 120 cm., thereby casting light upon it of relative intensities = approx. 32, 16, 8, 4, 2, 1, respectively. The latter figures are the 'light intensities' adopted throughout the present paper.

The circuit, recording apparatus, and calibration apparatus were arranged as in Fig. 2. The leaf was in series with two mirror-galvanometers (only one shown in figure) and a potentiometer. One galvanometer was used for making records, the other for observations. The recording galvanometer reflected upon a horizontal slit in the side of a box. Behind the slit was a piece of bromide paper 15 by 6 inches. The passage of the paper past the slit occupied about 2½ hours. The drum was driven by the friction of a cord drawn by a weight and maintained at constant speed by clockwork. A time-signal of sufficient accuracy was provided by casting a beam of light upon the edge of the slit at any required interval. For shorter experiments (up to 40 minutes) another recording apparatus was used. In this a photographic plate (4¼ by 3¼ inches) was let down by clockwork past a horizontal slit.

The recording galvanometer was of the d'Arsonval pattern, made by

Elliott, of 1,800 ohms resistance, and giving a deflexion of 1 mm. at a distance of 50 cm. with a current of 8.6×10^{-10} ampere. It was dead-beat. The observation galvanometer was also of the same type but less sensitive. It was used for preliminary observations and for obtaining the values of electrical effects which might happen to be beyond the range of the recording apparatus.

The potentiometer was used for calibrating galvanometric records in terms of E.M.F. With a Leclanché cell of 1·4 volts, the constant resistance
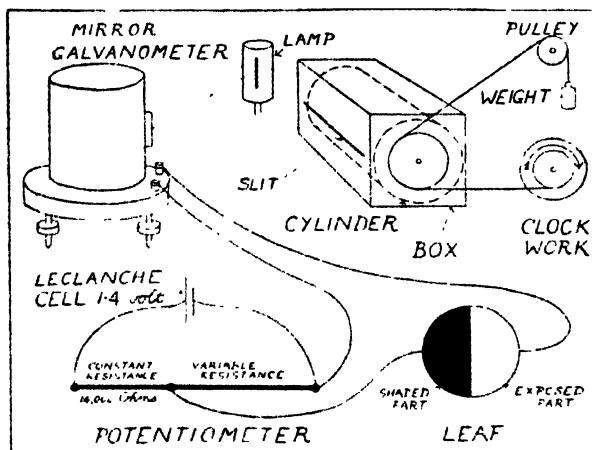
Fig. 2. Arrangement of apparatus. The circuit, calibration apparatus (potentiometer), and recording apparatus are indicated. For details see text.

14,000 ohms, and the variable resistance, for example, 100 ohms, a P.D. would be introduced into the main circuit of $\dfrac{1.4 \times 100}{14,000 + 100}$ = approx. 0·01 volt ($=$ 10 millivolts).

(b) *Reading of the records.*

N.B. In the photographic records, unless otherwise stated, *upward* displacement of the tracing indicates *positivity* of the exposed part of the leaf (i. e. current in the leaf from shaded to exposed part, in the galvanometer exposed to shaded). *Downward* displacement of the tracing indicates *negativity* of the exposed part.

The system of recording will best be understood by an example. Fig. 3 shows the response of a young leaf of cabbage (*Brassica oleracea*) cut from a plant which had been growing in a greenhouse. The electrodes were placed upon the lower surface, symmetrically on either side of the midrib, and 1·3 cm. apart.

The portions *ab* and *gh* of the tracing were obtained while the recording galvanometer was short-circuited. A line *abgh* therefore is the zero.

(The portions *ab* and *gh* are omitted in all other records reproduced in this paper.)   At *b* the leaf is put into circuit with the galvanometer.   As shown by the upward displacement of the tracing there is a small current in the leaf which is gradually subsiding.   This may be called the 'initial current'.

(c) *The 'initial current'.*

When a tissue is connected by electrodes to a galvanometer, an electric current is almost invariably found to be present which will here be spoken of as the initial current—a non-committal term.   This current may evidently be due to various causes, and is constituted as follows: (*a*) of more or less
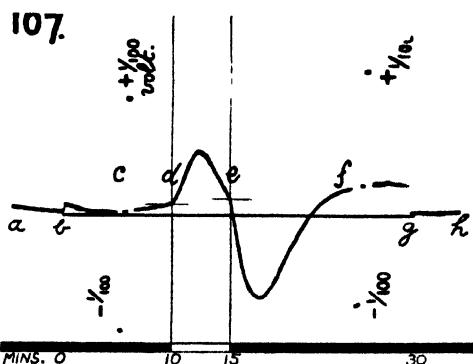
Fig. 3.   A typical record.   Electrical response of a leaf of cabbage to illumination lasting five minutes.

In this and all other records a period of illumination is shown by a white bar at the base of the record; periods of darkness are shown by black bars.

(*a*) The record starts, the galvanometer being short-circuited.   (*b*) Leaf and galvanometer are connected in circuit.   (*c*) Calibration.   '(*d*) The leaf is exposed to light and shows a *positive effect* which subsides.   (*e*) The light is cut off and the leaf shows a *negative after-effect.*   (*f*) Calibration.   (*g*) The galvanometer is short-circuited.   (*h*) The record ends.

quickly subsiding currents caused by mechanical disturbances while setting up the leaf for experiment, by previous exposure to light, &c., and (*b*) of long-persistent currents due to, e. g., unequal imbibition of water from the two electrodes and to unknown but natural causes within the plant.   These latter currents are said to be well marked if the electrodes are placed upon two unlike regions, e. g. midrib and mesophyll,[1] and may perhaps with justice be called 'normal' currents.

In my experiments, in which the electrodes were always placed as symmetrically as possible, the initial current followed no apparent rule either as to direction or magnitude.   Thus in Fig. 3 it is quite small (0·4 millivolt), subsides and then increases; in Fig. 4, second record, the behaviour of the current during some 30 minutes can be traced; in Fig. 5, middle record, the current is exceptionally large (13 millivolts).

[1] Kunkel, A., Arb. Bot. Inst. Würzburg, vol. ii (1897), pp. 1–17: 'Über elektromotorische Wirkungen an unverletzten lebenden Pflanzentheilen.'

So far as the present investigation is concerned the initial current appears to be of no significance, and, provided its subsidence (or augmentation) is not too rapid, there is in general no reason to wait till zero is reached
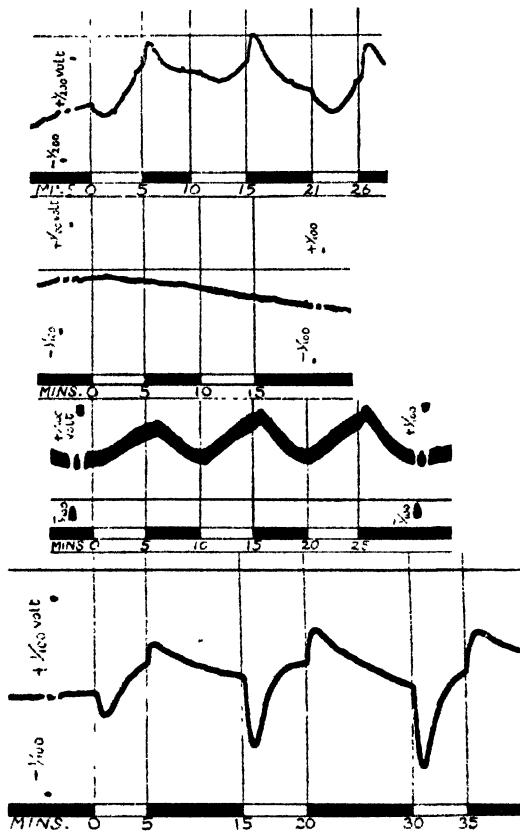
.FIG. 4. Photo-electric responses of *Hydrangea* petals at various stages of development.

Top record (No. 58) : Stage 1 : *Young green petal.* The effects are negative, the after effects positive.

Second record (No. 65) : Stage 2 : *white petal.* No response is given.

Third record (No. 28) : Stage 3 : *creamy petal.* Positive effects are shown which subside subsequently to illumination.

Bottom record (No. 36) : Stage 4 : *mature green petal.* As in Stage 1 the effects are negative, the after-effects positive.

before starting to make tests. It does not apparently influence the response to light (or other stimuli), for the response can, as it were, be superimposed upon the initial current.

(d) *Calibration.*

At the point *c* (Fig. 3) the current from 0·01 volt is passed through the

plant, first in one direction, then in the other.   This calibration is repeated towards the end of the record at *f.* By means of a ruler, the value of o·o1 volt is found = approx. 24 mm., either in the positive or negative direction. Thus a displacement of 1 mm. represents approx. o·4 millivolt.   The value is the same at the beginning and at the end of the record.

(e) *The photo-electric response.*

At *d* the shutter above the water bath is removed, and the exposed part of the leaf subjected to an illumination of five minutes ending at *e.* The *effect* of the light is shown in the upward displacement of the tracing. As already explained, this indicates a current in the leaf towards the illuminated part.   Thus the immediate effect of light is *positive.*   This effect is seen to reach a maximum and then to decline.   At the end of illumination *e* there is a pronounced *after-effect* in the *negative* direction, i. e. in the opposite direction from the original effect.

To calculate the effect, the maximum displacement of the tracing during illumination is measured, taking a horizontal line through *d* as zero. Similarly the after-effect is calculated by measuring the displacement of the tracing from the horizontal line through *e.*   The effect is 11 mm., the after-effect 20 mm., i. e. + 4·4 and − 8 millivolts respectively.

PROOF THAT THE CURRENTS ARE NOT DUE TO A RISE OF
TEMPERATURE OR TO EVAPORATION.

(a) *Rise of temperature.*

Light, even when deprived of all so-called heat rays, must cause a rise of temperature in any body which absorbs it.   If any such change of temperature is responsible for the photo-electric response of a leaf, then a similar effect should *a fortiori* be caused by heat.   That this is not the case is shown by the following comparison of the effects of heat and of light.

To obtain *heat without light,* a glass plate coated with black paint was substituted for the water bath, and all light carefully excluded from the leaf chamber.   A measure of the energy of the heat rays was given by a thermopile occupying the position of the leaf.   The energy was equivalent to 2·9 units.

To obtain *light without heat,* the water bath was filled to a depth of 6 cm., the lamp being at 21 cm. above the position of the leaf (i. e. the nearest position in which it was ever used in these experiments).   A layer of water 5 cm. deep was sufficient to cut off all heat rays, as indicated by the following observations:

Depth of Water in Bath.	*Energy of Radiation as measured by a Thermopile occupying the Position of the Leaf.*
2 cm.	2·7 units
3 ,,	2·3 ,,
4 ,,	2·1 ,,
5 ,,	2 ,,
6 ,,	2 ,,

Since an increase in the depth of water beyond 5 cm. did not appreciably decrease the amount of radiation passing through, it was concluded that this depth of water was sufficient to cut off all heat rays. For it is evident that an increase in the depth of water would not affect the passage of light to anything like the same degree as it would affect the passage of heat.

Thus in these comparisons, the energy of the heat rays used was roughly 45 per cent. in excess of that of the light.

Using a young leaf of cabbage, two five-minute exposures to *heat* rays caused no electric response. On the other hand, a one-minute exposure to *light* caused an immediate marked response exceeding 4 millivolts. Other tests such as this gave similar results.

Finally, concurrent observations were made of electrical changes and of temperature in the exposed portion of a leaf. The latter were measured by a fine thermo-couple inserted into a vein after the manner of Matthaei.[1]

Leaf exposed to :	Rise of Temperature during 5 Minutes' Exposure. °C.	Electrical Response. Millivolts.
Heat	+ 1·8	No distinct response
Light	nil	+ 20
Heat	+ 1·2	No distinct response
Light	nil	+ 22

It is therefore evident that the electrical changes observed in leaves under my conditions of experiment are brought about by the action of light, not of heat.

(b) *Evaporation.*

Evaporation from a moist surface is accompanied by electric currents. Thus, on exposure to the full light and heat from the electric lamp, a current of + 0·025 millivolt was produced in a boiled leaf of geranium and of − 0·5 millivolt in a piece of wet filter-paper.

But with the water bath in position and the lamp at 21 cm. distance, no photo-electric current could be detected in boiled leaves of cabbage and geranium. Thus, under my conditions of experiment, electrical changes due to evaporation need not be considered.

STUDY OF THE CURRENTS IN VARIOUS ORGANS.

(a) *Hydrangea petals (Hydrangea Hortensia).*

To indicate whether or not the photo-electric response depends on the presence of chlorophyll, the petals of *Hydrangea* were used. The suitability of these as test objects was suggested to the writer by Professor J. Bretland Farmer. These petals, which give *Hydrangea* its showy appearance, are

[1] Matthaei, G. L. C.: Phil. Trans. Roy. Soc., B., vol. cxcvii, pp. 47-105, 1905. See p. 76.

in reality metamorphosed florets. They bear stomata upon the lower surface, but not upon the upper. While young they are of a yellowish green, but as they enlarge they lose the green colouring and become pure white. In some plants they become pink or blue instead of white. But at a later stage the petals regain their green colour, and this persists until the flowers fade.

Parallel with these stages of coloration, characteristic differences of photo-electric response are revealed.

The observations which led to this conclusion are summarized in Table I, and selected records are shown in Fig. 4.

The stages are as follows:

Stage 1. Petal yellowish green. At this stage of development a photo-electric response was given, the effect being negative and the after-effect positive.

Stage 2. Petal pure white (or pink or blue). At this stage there was no trace whatever of electrical response to light. One exception was noted in which there was a very slight fluctuation at the beginning and at the end of illumination.

Stage 3. Petal white with a creamy or greenish tinge, i.e. just entering upon the final stage of green. In this case there was a photo-electric response, but of a different nature from that of green petals (whether young or old), the effect being positive, the after-effect negative. This is the reverse of the corresponding changes in green petals.

It may be mentioned that both types of response are of a physiological nature, for it was found that treatment of petals with chloroform vapour completely abolished their power of response in twenty to forty minutes.

Stage 4. Petal at its mature stage of green. A photo-electric response is shown similar to that of Stage 1 (the young green petals), i.e. effect negative, after-effect positive.

(b) *Petals, leaves, &c., green and non-green.*

Chiefly during May and June 1924 a number of green and non-green parts of plants were tested. In each case a record was taken. In records which lasted for less than twenty minutes it was not thought necessary to keep the stalks of leaves &c. in water during experiments.

In case absence of response in a petal should merely be due to poor condition, the flowers were chosen with especial care, just before they were fully expanded, and cut in some cases from pot plants brought to the laboratory. Generally also a leaf, chosen from the same stem or plant as the flower, was tested for comparison.

The observations are summarized in Table II. It will be seen that marked responses were given by the green leaves of wallflower, primrose, pansy, *Anemone, Aubretia, Clematis,* and buttercup, whereas petals gathered

from flowers upon the same actual plants' gave no response or at most mere traces too small to measure. Striking contrasts in behaviour were also shown between green leaves and albino leaves, and also by petals or petaloid organs similar to each other except in regard to green colouring : for example, a pure yellow daffodil petal gave no response, while a green petal gave a marked response.

The only non-green organ to give a fair response was a petal of *Phyllocactus* (Record 30). But, as learnt from the record, even this was of a wavering nature, quite unlike the marked and comparatively rapid reactions shown generally by green leaves. It might be accounted for by some phototropic or unknown photo-chemical reaction, but in view of the fact that the petals of this plant frequently show a green colouring, it seems not unlikely that a small amount of chlorophyll may have been concealed by the red pigment of the petal and would thus account for the result.

That the response is a general property of green leaves has been indicated to me by the study of some fifty species, including herbs, shrubs. trees, and succulents, all of which responded. These tests were done with young leaves. Nevertheless A. D. Waller (2) failed to obtain response from ‘ the leaves of ordinary garden shrubs and trees ’.

(c) *Direction of the photo-electric current in various leaves.*

Leaves of *Iris* and *Nicotiana* were found by A. D. Waller to give negative effects and positive after-effects, whereas *Tropaeolum* and *Matthiola* gave positive effects and negative after-effects.

By the study of different kinds of leaves under various conditions it has become apparent to me that this curious contrast in behaviour is determined partly by the specific nature of the leaves, partly by the conditions to which they have been subjected. Thus cabbage leaves have invariably given positive effects and negative after-effects (e. g. Fig. 3). *Tropaeolum majus* (nasturtium) acts in the same way, especially if the leaf has been in darkness for some hours previously to testing (e. g. Fig. 6) ; but if the leaf is brought into the laboratory from a sunny position and then tested, the main positive effect is preceded by a brief negative deflexion ; similarly the main negative after-effect is preceded by a brief positive deflexion. On the other hand, *Pelargonium zonale* (garden geranium) has given negative effects and positive after-effects in a prolonged series of tests (e. g. Fig. 5) ; nevertheless it was found possible to induce a leaf to reverse the directions of effect and of after-effect by previously keeping it in darkness for an abnormal length of time.

So far as my observations have gone, the leaves of woody plants (Rosaceae, *Rhododendron*, oak, &c.) and of Monocotyledons give, like *Pelargonium*, pronounced negative effects ; on the other hand, leaves of
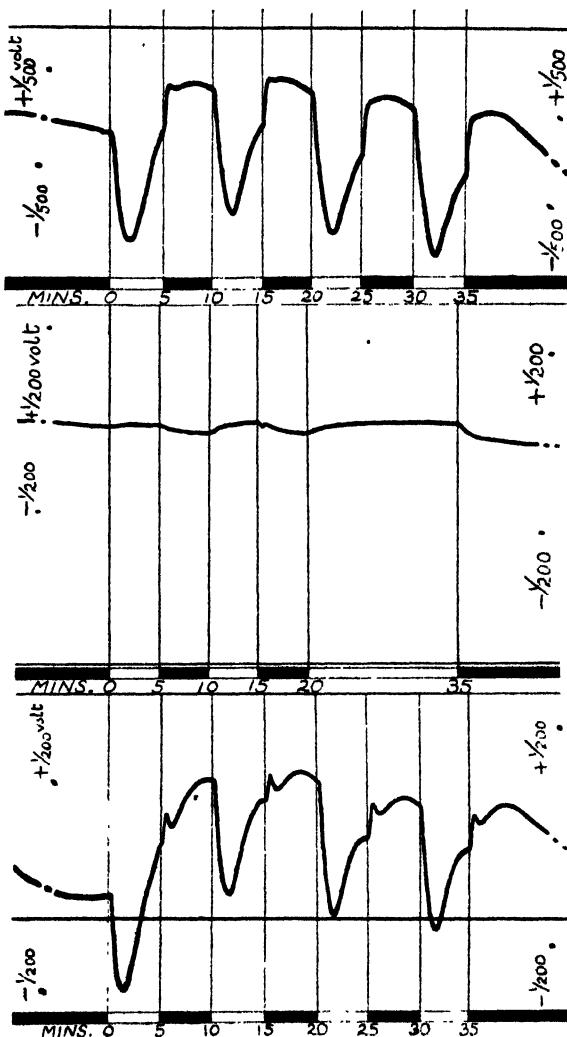
FIG. 5. Photo-electric responses of *Pelargonium zonale*, var. ' Flower of Spring'.

Records taken on the same day of three leaves gathered from a single plant.

Top record (No. 101) : *Green leaf*, partially exposed. The effects of illumination are to render the exposed part of the leaf negative to the shaded part. The after-effects are positive.

Middle record (No. 102) : *Albino leaf*, partially exposed. The responses are comparatively feeble, and in the opposite sense from the other two records.

Lower record (No. 103) : *Variegated leaf*, whole surface exposed. The effects of illumination are to render the green part negative to the albino part. The after-effects are positive.

The last two responses in the middle record were obtained with double the intensity of light employed for all the other responses.

Cruciferae, Papilionaceae, Solanaceae, and other leaves of the starch-forming class resemble *Tropaeolum* in their tendency to become positive on illumination.

The conditions which determine the direction of these photo-electric currents are being made the subject of further analysis (12, 13).
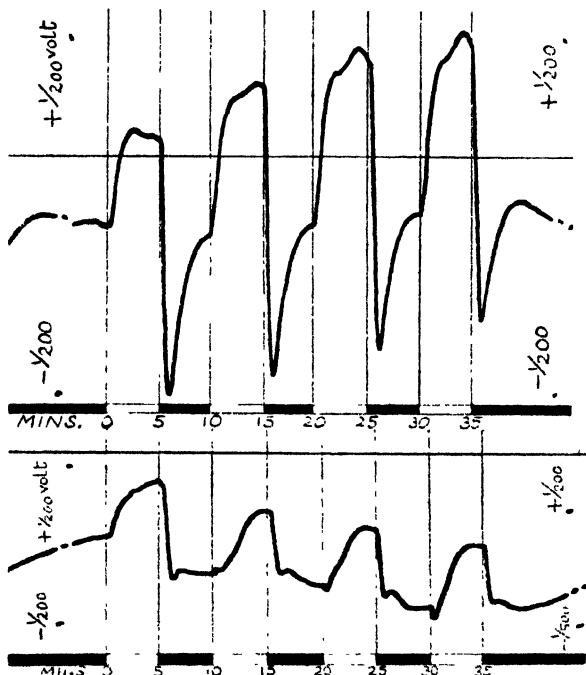
FIG. 6. Photo-electric responses of *Tropaeolum maj us.*
Top record (No. 42) : *Green leaf,* partially exposed.
Lower record (No. 77) : *Variegated leaf,* whole surface exposed.
Effects are positive, after-effects negative. Cf. *Pelargonium,* Fig. 5.

(d) *Variegated leaves.*

In all cases of photo-electric response so far mentioned the differences of potential have been measured between an illuminated portion of the leaf and a shaded (control) portion at relatively constant potential. But in variegated leaves, whose tissue is broken into patches of green and white, there exists a *natural* mechanism for the establishment of such differences of potential. For since, as already shown, albino tissue is not affected by light, or at least very little affected, it should come to the same thing whether we measure the difference of potential between an illuminated green tissue and a shaded green tissue, or between a green tissue and an albino tissue both illuminated. In the latter case we simply place one

electrode upon an albino area, the other electrode upon a green area, and leave the *whole* surface of the leaf unshaded. The circuit is so arranged that positivity of green tissue in relation to albino tissue is indicated by upward displacement of the record and negativity by downward displacement.

By either method—green leaf partially illuminated or variegated leaf with whole surface exposed—the contrast in behaviour between the green tissue of *Pelargonium* and that of *Tropaeolum*, previously pointed out, can be made manifest. A study of the records in Figs. 5 and 6 will make this clear.

This conclusion has been confirmed for *Pelargonium* by records of 10 green leaves (30 exposures to light) and 10 variegated leaves (41 exposures), in addition to the data concerning albino leaves and green leaves at the beginning of Table II. In the case of *Tropaeolum* confirmatory observations have been made upon 11 green leaves (71 exposures) and 5 variegated leaves (28 exposures).

It may be mentioned that no pure albino leaves were found on the particular plants of variegated *Tropaeolum* which I grew.

Professor F. E. Weiss, who originally suggested the use of a single plant bearing the three kinds of leaves mentioned, kindly sent me plants of variegated *Arabis*, but the specimens of variegated *Pelargonium* which I subsequently obtained proved better subjects for experiment.

(e) *Holly (Ilex Aquifolium).*

Leaves of holly were found to have an electrical resistance so high that no flow of current could be detected in them. This is no doubt due to their richness in sclerotic tissue. The resistance is greatly reduced after passage through the leaf of one or two induction shocks from a coil, and the state of diminished resistance persists for an indefinite time. It was found that during this time photo-electric responses could be observed in the same manner as with other leaves. Thus young leaves, gathered from a holly tree in July and treated in the manner described, gave results similar to *Pelargonium*, viz. an albino leaf gave no response, while the green tissue of a variegated leaf and also of a normal leaf (partially shaded) gave negative effects and positive after-effects.

(f) *A liverwort (Pellia epiphylla).*

Records show that a frond gave well-marked responses, though the values of these, expressed in voltage, are comparatively small (see end of Table II).

The interest of the results lies in the fact that the object tested was of so simple a structure. It consisted of a green translucent plate 20 mm. long by 7 mm. wide, of uniform parenchyma and without any system of inter-

cellular spaces. A few rhizoids were present upon the median region of the lower surface. Stomata of course were absent. Nevertheless, the records, both with short and with prolonged illumination, are not unlike what might have been obtained with the leaves of various Angiosperms.

Thus it is proved that the manifestation of a photo-electric current is not dependent upon the presence of stomata or other complex tissue systems characteristic of the higher plants.

(g) *Etiolation.*

A petiole, chosen from the centre of a blanched celery plant, gave distinct positive effects, thus confirming the statement of Bose (8, p. 396). No chloroplasts could be detected by microscopic examination. Seedlings of mustard and of broad bean were therefore raised in darkness and then tested. In this case, however, the etiolated leaves gave little, if any, response. Yet when the chlorophyll had been allowed to develop through exposure to sunlight, normal photo-electric responses were shown. With broad beans series of records were obtained showing a gradual development of the power of response as the leaves developed to their full green colour. This was a striking demonstration.

It appears probable, therefore, that blanched celery, whose early stages of growth were passed under normal conditions of light, possesses photochemical properties not present in plants whose entire development has been in darkness. *Hydrangea* petals at 'Stage 3' (see above) give a response somewhat like that of blanched celery.

MODIFICATION OF THE CURRENTS BY CERTAIN CONDITIONS KNOWN TO INFLUENCE PHOTOSYNTHESIS.

Since the photo-electric response, as above shown, is in the main dependent upon the activity of chlorophyll, and since photosynthesis is the activity most characteristic of chlorophyll, we may expect that those conditions which influence photosynthesis will also have an effect upon the photo-electric response.

It will now be shown that, so far as experiments have gone, this expectation is fulfilled.

(a) *Light through glass of various colours.*

I assured myself of the marked differences of response to such variations of illumination by means of 'Ilford spectrum glasses'. With the red glass an effect of + 3 millivolts was given, with the orange + 1·2, while with yellow, yellow-green, green, blue-green, blue and violet, the effects did not exceed 0·6 (Record 79, *Tropaeolum*).

Owing to the unequal intensities of light supplied through the different

glasses, these observations give no comparison of the relative efficacy of rays of different wave-length.   They do, however, show a parallel with the well-known effects upon photosynthesis demonstrated by means of 'Sachs's double-walled bell-jars' and other devices.

A comparison of the electrical response to different coloured lights of equal intensity (energy) appears to me an attractive field for investigation.

(b) *Increased supply of carbon dioxide* (CO_2).

While verifying the fact that an increased supply of CO_2 causes augmentation of photo-electric response, I found that leaves undergo wide variations of reactivity : thus a leaf of cabbage whose response in normal air was 17 millivolts gave a response of doubled magnitude in air containing 5 per cent. of CO_2; whereas a leaf which gave a smaller response to start with (4·4 millivolts) showed in the enriched atmosphere a more than five-fold increase of response.

I already have indications that such variations of behaviour will become explicable in the light of the principle of ' limiting factors ' introduced into plant physiology by F. F. Blackman.

(c) *Carbon dioxide-free air.*

In the case of *Begonia*, A. D. Waller (5) demonstrated modification of response of a leaf after seven hours in a stream of CO_2-free air, followed by a return to the normal in ordinary air.   His tests were done with short illuminations, i. e. five minutes generally.

The difficulty of detecting modification of the response through deficiency of CO_2 is explicable when we consider the impossibility of freeing the leaf tissue itself of the products of respiration.

It might be expected that by continued exposure to light this internal store of assimilable material would be depleted, and I am able to state that comparisons of the electrical changes in leaves of cabbage during illuminations lasting forty to sixty minutes in a stream of normal air and in a stream of CO_2-free air have given clear indications of a modification of response attributable to deprivation of CO_2.

The results will be given in connexion with the effects of prolonged illumination to be dealt with in a future paper.

CONCLUSIONS.

1. The well-marked photo-electric response shown by various leaves and other green parts of plants is mainly or entirely dependent upon the metabolic activity of chlorophyll : electric currents associated with photo-tropic or other reactions to light, if existent in such organs, being quite obscured by those dependent on chlorophyll.

2. The direction of the photo-electric current varies in different leaves, and is determined partly by the specific nature of the leaf, partly by the conditions to which the leaf has been subjected.

3. Photo-electric currents may occur in the absence of chlorophyll, as shown in the cases of *Hydrangea* petals and of blanched celery.

SUMMARY.

A critical survey is given of previous work concerning photo-electric currents in plants.

Apparatus and method are described in detail.

The chief experimental results in this paper are as follows :

1. The petals of *Hydrangea* during development undergo parallel changes in coloration and in photo-electric response. The young petals are green and give electrical response to light ; at a later stage the petals lose their green colour and also their power of response ; finally the green colour returns to the petals and also the power of response.

During the very early stages of re-formation of the green pigment in the mature petal a response is given, but of a different type from that shown by green petals. A somewhat similar response has been observed in blanched petioles of celery. These are the only cases observed of response to light in the absence of chlorophyll.

2. A number of green and non-green petals and albino and green leaves from various plants have shown that the power of electrical response to light is in general confined to those organs which show a green colouring.

3. The direction of the photo-electric current in different classes of leaves subjected to various conditions is briefly dealt with.

4. Instead of measuring the difference of potential between illuminated and shaded portions of a green leaf, we may measure the difference between a green and an albino portion of a variegated leaf. Thus *either* shaded green tissue *or* unshaded albino tissue may be used as a control at relatively constant potential.

5. By both of these methods it was found that the green tissue of leaves of *Pelargonium zonale* becomes negative on exposure to light, positive when the light is cut off. On the other hand, *Tropaeolum majus*, tested in the same way, as a rule becomes positive on exposure and negative when the light is cut off.

6. A green, an albino, and a variegated leaf of holly gave results similar to those of *Pelargonium*, but owing to their high electrical resistance no current can be detected in normal leaves. The resistance can be lowered by passage of an electric shock, after which it is possible to detect the photo-electric response.

7. Photo-electric responses have been observed in the case of a simple liverwort, *Pellia epiphylla*, thus showing that the manifestation of the

response is not dependent on the presence of stomata or other complex structures characteristic of the higher plants.

8. Seedlings raised in darkness gave little if any response, but when the chlorophyll was allowed to develop by exposure to sunlight, the plants acquired the normal power of response.

9. An example is given showing that light through red glass causes a larger response than light through other coloured glasses.

10. Modifications of the response through increased and decreased supplies of CO_2 are reported and discussed.

The evidence for associating the photo-electric currents of green leaves with the activity of chlorophyll may be briefly set out as follows : (*a*) The correlation between photo-electric response and chlorophyll-content of *Hydrangea* petals during development. (*b*) General absence of the response in non-green petals and in albino leaves. (*c*) The similarity of behaviour of albino parts of variegated leaves and of shaded parts of green leaves. (*d*) Augmentation of the response consequent upon increased supply of CO_2. (*e*) Modification of the response through subjecting a leaf to CO_2-free air ; recovery on admission of normal air. (*f*) Larger response to light through red glass than to light through other coloured glasses, recalling the well-known experiments of Draper, Sachs, and others on photosynthesis by different coloured lights. (*g*) Gradual development of the power of response and of green colour in etiolated seedlings after exposure to sunlight.

Finally I would like to make clear that the line of investigation of which this paper marks the first stage took origin entirely through the teaching and inspiration of my father, the late Professor A. D. Waller.

TABLES I and II.

In the case of green leaves, albino leaves, and petals the signs + and — signify respectively positivity and negativity of an exposed part as compared with a shaded part of the organ. In the case of a variegated leaf the signs refer respectively to positivity and negativity of a green part of the leaf as compared with an albino part, the whole surface being left unshielded.

Length of illumination in nearly all cases was five minutes, though in a few cases it varied up to seven minutes or down to two minutes: these variations are of no significance as regards the conclusions to be drawn from the tables.

The numerical values of response are those obtained at the first test in any particular record, and differ from those obtained in later tests. Later tests, however, were without exception confirmatory of the results obtained in first tests.

TABLE I. *Photo-electric responses of Hydrangea petals at various stages of development.*

Stage of Petal.	Date. 1923.	Room Temp. °C.	Record No.	Intensity of Illumination.	Response Millivolts. Effect.	After-effect.	Number of Confirmatory Tests.
Young green	3 July	17·5	38	16	− 0·6	+ 0·5	1
	26 July	17·5	58	,,	− 1·1	+ 2	2
	30 July	17	62	,,	− 2·5	+ 1·7	1
White	30 June	16·5	34	,,	nil	nil	—
	3 July	17	37	,,	nil	nil	2
			37 [1]	,,	trace	trace	1
	26 July	18·5	60	,,	nil	nil	1
	30 July	17	63	,,	nil	nil	1
	31 July	17	65	,,	nil	nil	1
Blue	,,	17	66	32	nil	nil	—
Pink	,,	17	67	.,	nil	nil	—
White just turning green	23 June	17	28	8	+ 5·8	− 5·4	2
	30 June	17	33	16	+ 6·5	− 3	1
	,,	16	35	,,	+ 3	− 5	1
Mature green	23 June	17	27	8	− 3·2	+ 1·4	2
	30 June	17	32	16	− 1·7	+ 1	2
		16·5	34	,,	− 4	+ 6·4	2
	3 July	17	36	,,	− 2·1	+ 1·8	2
	26 July	19	61	,,	− 1·9	+ 1·3	1
	27 July	17	64	,,	− 2	+ 1·5	1

[1] The same petal as the preceding one, but with illumination on a different part.

TABLE II. *Various Leaves, Petals, &c. Response and Absence of Response to Light.*

NOTES.—The word 'petal' is used to denote any petaloid perianth segment.
Light intensity used throughout = 32.
In comparative tests, e.g. between a green and non-green petal from a single flower, the distance between the electrodes in the two cases was made as nearly as possible equal.
In the two right-hand columns, when a number in brackets precedes another number, e.g. in Record 160, $(-0.8)+14.3$, this means that a small negative deflexion preceded the main positive effect. Both deflexions are measured from the same base-line (see under 'Method').

Plant.	Date.	Room Temp. °C.	Record No.	Plant Organ.	Response Millivolts. Effect.	After-effect.
Pelargonium zonale	1924					
	2 May	17	145¹	albino leaf	nil	−1.2
	”	”	146¹	green leaf from same plant as 145	−0.6	+1.1
	19 May	”	185¹	green leaf	−1.4	+1.6
	6 June	16	186¹	albino leaf from same plant as 185	nil	nil
	”	”	190	green leaf	−6.3	+.3
	10 June	15.5	191¹	albino leaf from same plant as 190	trace	trace
	”	”	198	variegated leaf	−4.4	+6.6
	”	”	199¹	albino leaf from same plant as 198	nil	nil
Phyllocactus, sp.	1923					
	26 June	17.5	30¹	petal (scarlet)	−0.7	+0.8
	27 June	16	31¹	another petal from the same flower	no distinct response	
Begonia, sp.	30 Aug.	”	98¹	petal (white)	nil	nil
	”	”	99¹	petal (scarlet)	nil	nil
	”	”	100¹	petal (yellow)	nil	nil
Cheiranthus Cheiri	1924					
	3 May	15.5	147¹	petal (brown-red)	trace	trace
	”	16	148	leaf from same stem as 147	+9.5	−10.5
	4 May	15	159¹	petal (yellow)	nil	nil
	”	”	160	leaf from same plant as 159	$(-0.8)+14.3$	−(not measured)
Narcissus Pseudo-narcissus	3 May	16	149	green outer petal	−8.5	+6.8
	”	”	150¹	part of yellow corona from same flower	nil	nil
	”	”	152¹	leaf from same plant	−5	nil
	”	”	151¹	pale yellow petal from another flower	nil	nil
	”	”	153¹	leaf from same plant as 151	−4.4	+trace

Plant	Date		Ref.	Description		
Primula vulgaris	4 May	15.5	154[1]	petal	nil	nil
	,,	16	156[1]	leaf from same plant	-2.8	$+0.5$
Viola tricolor	7 May	14	161[1]	leaf	$(-1.8)+4.2$	$(+4.5)-2.7$
	,,	14.5	162	petal (purple) from same plant	nil	nil
Tulipa, sp.	,,	16	163	outer petal (red with green stripe[2])	-0.2	$+0.2$
	,,	,,	164[1]	inner petal (red) from same flower	$-$trace	$+$trace
Anemone coronaria	8 May	15	165[1]	petal (pink with shade of green)	-2.3	$+3.6$
	,,	,,	166[1]	petal (purple)	nil	nil
	,,	,,	167[1]	leaf from involucre of 166	$(-1.4)+1.8$	$(+0.2)(-0.1)+1$
Aubretia purpurea	10 May	16.5	169[1]	leaf	$(-2)+11.2$	$(+0.2)-13.6$
	,,	,,	170[1]	petal (mauve) from same plant	nil	nil
Iris Xiphium	12 May	18	172[1]	petal (blue-mauve with green in central region[2])	-4.8	$+3.4$
	,,	18.5	173[1]	petaloid stigma-lobe (blue-mauve) from same flower	nil	nil
Rhododendron, sp.	,,	19	174[1]	petal (dark pink) cut from corolla tube	nil	nil
Scilla nonscripta	,,	18.5	175[1]	mature white petal	nil	nil
	,,	18	176[1]	petal from bud on same inflorescence; greenish tinge	nil	nil
	,,	,,	177[1]	peduncle (pale green) of 175 and 176	$+0.8$	-0.2
Clematis, sp.	17 May	17	179[1]	green leaflet	-7.6	$(+0.4)(-0.4)+5.4$
	,,	18	180[1]	petal (pale mauve) from same stem	nil	nil
Ranunculus repens	19 May	16	183[1]	portion of a leaf	-1	$(+0.2)(-0.5)+1.7$
	,,	,,	184[1]	petal (yellow) from flower on same plant	no distinct response	
Paeonia, sp.	7 June	16	192[1]	green sepal	-5.7	$+7.2$
	,,	,,	193[1]	petal (red) from same flower	nil	nil
Pellia epiphylla	5 Feb.	16	118[1]	frond	-0.5	$+0.1$
	,,	14.5	119	same frond (illumination 1¾ hours)	-0.8	$+1.3$

[1] In these cases a confirmatory test was made.
[2] Electrodes on green tissue.

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[1] On p. 1094 of this paper the following values for responses are given :—

Blanc	0·0075 volt
Bleu	0·0075 ,,
Rouge	0·0070 ,,

The value given for blue is a misprint. In the copy preserved among the personal papers of the late Professor A. D. Waller the figure was altered in his handwriting to 0·0025 volt.

The Origin and Course of Vascular Bundles in Achyranthes aspera, L.

BY

R. H. DASTUR.

With seven Figures in the Text.

INTRODUCTION.

MANY dicotyledonous plants and a few Gymnosperms differ in their bundle system from the rest in the arrangement of the vascular tissue in the stem in that the bundles do not form a single ring. In addition to a ring of bundles arranged according to the usual type extra bundles are found in the pith within the ring or in the cortex outside it. In some cases the bundles are arranged in several concentric circles, or are so arranged that in a transverse section they appear irregularly scattered, with the exception of the most peripheral ones which form a ring. The most remarkable exceptions to the main type occur in species of *Begonia,* and among the Umbelliferae, Nymphaeaceae, Calycanthaceae, and many other families.

The arrangement of the bundles in these cases is either due to the oblique radial course of the bundles of the leaf-trace, or to the presence of cauline bundles in addition to the bundles of the leaf-trace which are arranged in a typical ring.

In Nyctaginaceae, Amarantaceae, Piperaceae, and other families, the anomalous arrangement of the vascular bundles is due to the radial oblique course of the leaf-trace bundles. Some of these bundles entering the stem are arranged in a ring, and have a radially perpendicular course in it. But others passing farther inwards are scattered through the pith or are arranged in rings.

Though the peculiarities in the stem structures and the different methods of anomalous secondary thickening have been investigated by Link (2) and Sanio (4) in the plants of the above-mentioned families, the

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course of vascular bundles has been worked out in a very few cases. De Bary (1) has described the course of vascular bundles in *Mirabilis Jalapa*, and has traced the course of medullary bundles inside the extra-fascicular ring. Link (3) and Unger (5) have also traced the course of vascular bundles in *Amaranthus retroflexus, Amaranthus caudatus*, and *Exolus*
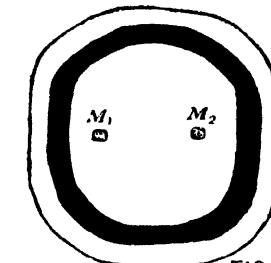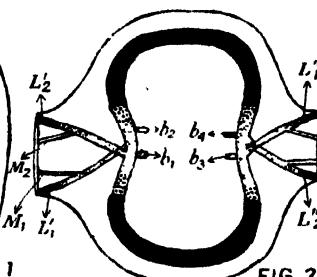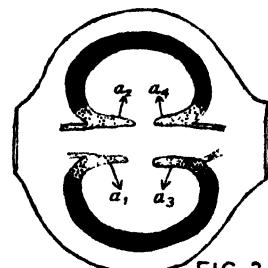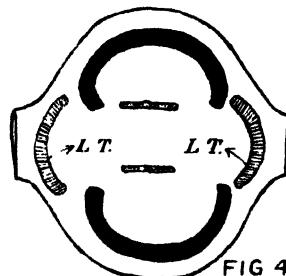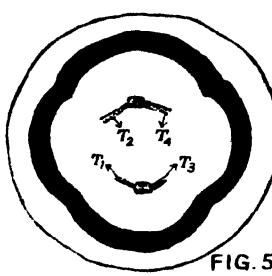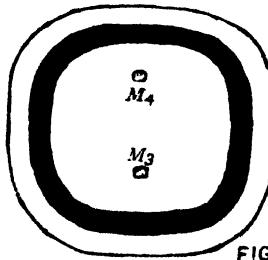
Fig. 1.   Transverse section passing through a young internode of *Achyranthes aspera* Linn.
Fig. 2.   Transverse section showing the two lateral parts of the ring curving inwards, the bifurcations of the medullary bundles of the stem and branches and the bifurcations of the rings of the branches.
Fig. 3.   Transverse section showing the bifurcations nearing one another from two opposite sides of the ring of the stem.
Fig 4.   Transverse section showing the fusion of bifurcations and the fresh arches of the leaf-trace bundles filling the gaps in the ring.
Fig. 5.   Transverse section showing the completion of the ring and the bifurcations from the leaf-trace bundles joining the newly formed medullary bundles.
Fig. 6.   Transverse section showing the newly formed bundles of the next internode.
(For explanation of the lettering see end of paper.)

marginatus, and have shown how several medullary bundles are formed within the rings. A similar arrangement has been observed by the writer in *Amaranthus spinosus*, L., and *Amaranthus polygamus*, L., but on investigating the course of the leaf-trace bundles in *Achyranthes aspera*, L. a novel and interesting feature was noticed which is quite different from those described in the other cases mentioned above. Wilson (6) has recently studied the anatomy of Chenopodiaceae and Amarantaceae, and has described the course of medullary bundles in some plants of the two families. But no mention has been made by him of the unique features in the course of medullary bundles in *Achyranthes aspera*, L.

INVESTIGATION.

Achyranthes aspera, L., is an annual which is common as a weed on waste ground throughout the Bombay Presidency. The stem bears opposite decussate leaves with secondary branches in their axils, which in turn give rise to leaves and branches of the third order in the same manner as the main stem. The flowers appear in August in long spikes terminating the stem and branches.

The stem undergoes an anomalous form of secondary thickening by the formation of cambium layers in centrifugal order after the activity of the normal cambium ring ceases. A transverse section passing through a young internode shows the normal ring of vascular bundles, inside which are seen two medullary bundles situated diametrically opposite to one another (Fig. 1, M_1 and M_2). The position of these two medullary bundles remains the same throughout the length of an internode as they traverse it with a radially perpendicular course. But in the next internode a transverse section shows that they do not occupy the same position, but are now placed in a plane at right angles to the former (Fig. 6, M_3 and M_4). This change in the position of the medullary bundles occurs regularly in the internodes of the main stem and branches. Series of transverse and longitudinal sections disclose the following further details.

The normal ring of vascular bundles is derived from the bundles of the leaf-trace entering the stem from the leaves at the nodes. And as the arrangement of the leaves is decussate, two arches of bundles enter from the two leaves at each node (Figs. 4 and 7, *L.T.*). If the normal ring is regarded as composed of four parts, two lateral, one ventral, and one dorsal, the two lateral parts of the ring are made up of the two arches from the two leaves at one node, and the dorsal and ventral parts are made up of two arches from the two leaves at the next node. The two arches of bundles entering at each node traverse with a perpendicular course two internodes, and at the end of the second internode they are replaced by fresh arches of vascular bundles from the pair of leaves which are exactly below the upper pair of leaves.

The question arises, What happens to the bundles of the ring which are replaced by fresh ones after traversing two internodes? The bundles of the two lateral parts, or of the dorsal and ventral parts of the ring, as the case may be, at the end of the second internode curve inwards and each of the two opposite parts bifurcates (Fig. 7, a_1, a_2, a_3, and a_4). The two bifurcations a_1 and a_3 from the two opposite lateral sides join together on the ventral side (Fig. 7), and the other two, a_2 and a_4, on the dorsal side. They then pass down as the two medullary bundles M_3 and M_4 of the next internode below. At the end of the latter internode the ventral and the dorsal parts of the vascular ring will behave similarly, and will give rise

to another pair of medullary bundles which will be orientated at right angles to the former pair.

The gaps in the normal vascular ring thus produced on two opposite sides by the peculiar course of vascular bundles are filled in by the leaf-trace bundles entering from the two opposite leaves at the nodes (Fig. 7, *L.T.*), and the normal ring is again formed. At the same time a part of the freshly entering leaf-trace curves inwards, bifurcates, and the two bifurca-
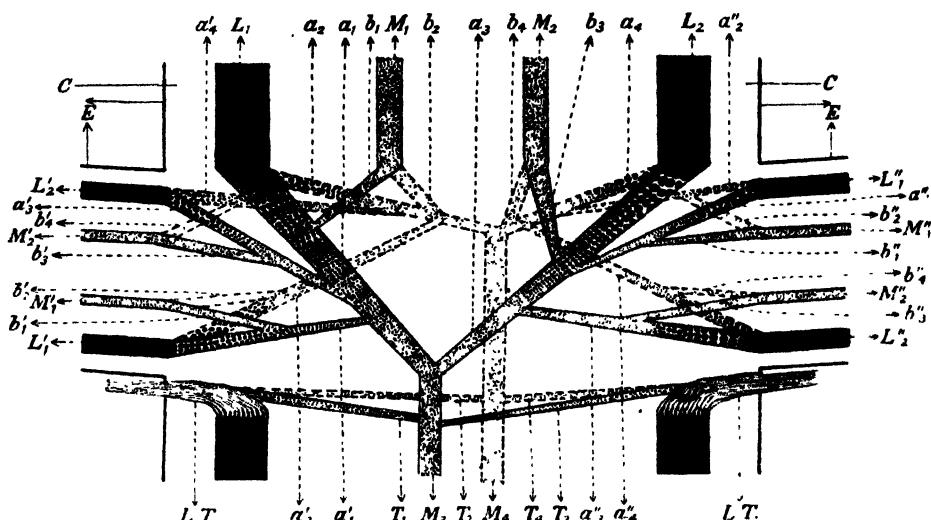
Fig. 7. Diagram illustrating the course and origin of the medullary bundles at the nodes. (For explanation see the text.)

tions from each side join the two newly formed medullary bundles of the next internode (Fig. 7, T_1, T_2, and T_3, T_4).

The vascular rings of the branches are similarly formed and the medullary bundles arise in the same manner. At a point where a secondary branch joins the main stem, the vascular ring of the branch is continuous with that of the stem on two opposite sides. The two remaining parts of the ring of the branch, just like that of the stem, curve inwards, bifurcate, and join the corresponding bifurcations of the vascular ring of the stem, giving rise to new medullary bundles (Fig. 7, a'_1, a'_2, a'_3, a'_4, and a''_1, a''_2, a''_3, a''_4). The vascular rings of the tertiary branches similarly give rise to their medullary bundles.

The medullary bundles traverse an internode with perpendicular course, and at the end of the internode they also bifurcate (Figs. 2 and 7, b_1, b_2, b_3, and b_4). The two bifurcations b_1 and b_2 of the medullary bundle M_1 join with the two bifurcations a_1 and a_2 of the vascular ring of the stem before the bifurcations of the latter fuse to form new medullary bundles.

The medullary bundles of the branches, like those of the stem, after traversing an internode with a perpendicular course, bifurcate, and the four bifurcations (Fig. 7, b'_1, b'_2, b'_3 and b'_4, or b''_1, b''_2, b''_3 and b''_4) thus produced join with the four bifurcations (a'_1, a'_2, a'_3, a'_4, or a''_1, a''_2, a''_3, a''_4) of the normal vascular rings.

Medullary bundles similarly developed could also be made out in the lower part of the inflorescence axis, but towards the apex they could not be distinguished as the tissues are not differentiated.

At the base of the stem the two medullary bundles curve inwards and meet in the centre of the stem. The phloem disappears before the fusion takes place. The fused mass of the xylem elements ends in the woody cylinder of the root.

DISCUSSION.

The medullary bundles which are formed in the manner described above are directly connected with the vascular bundles of the stem, branches, root, and leaves. They are also connected with one another, and with the medullary bundles of all the internodes. In this way a perfect communication is established between the elements of the whole conducting system.

In various species of *Amaranthus* so far investigated the medullary bundles are formed from the median bundles of the leaf-trace which penetrate farther inwards than the rest. These bundles, after traversing a few internodes, curve outwards and join with the bundles of the ring. But the course of the medullary bundles in *Achyranthes aspera*, L. becomes complicated on account of the fusion of the various strands which takes place in the formation of these bundles. Each medullary bundle is actually derived from the two bifurcations b_1 and b_3 from the upper pair of the medullary bundles M_1 and M_2, from the four bifurcations b'_1, b'_3, b''_1, and b''_3 of the two pairs of the medullary bundles M'_1, M'_2, M''_3 and M'''_4 of the two branches at each node, and from two bifurcations a_1 and a_3 from the two opposite sides of the normal ring of the stem, also from the four bifurcations a'_1, a'_3, and a''_1 and a''_3 from the normal rings of the two branches, and from two bifurcations T_1 and T_3 from the pair of opposite leaves at each node.

It is also evident that a definite relation exists between the arrangement of the leaves on the stem and the disposition of the medullary bundles inside the stem. Each pair of leaves at the node is at right angles to the pair of the medullary bundles of the internode below, and the relation between them is not obscured or destroyed by splittings or coalescences as is the case in many other plants.

In some cases such special phenomena of the distribution of the vascular bundles in the stems of an allied group of plants could be explained as a result of adaptation which has happened in a parent form through unknown causes during phylogenetic development, these peculiarities of

structure having persisted in the closely allied genera and species with some deviations.   These deviations are probably due to the different arrangement of the leaves on the stems.   The differences in the course and arrangement of the medullary bundles in *Achyranthes aspera*, L., and other plants of the family could be explained on the above suppositions.

SUMMARY.

1. In *Achyranthes aspera* each internode of the stem and branches contains a normal ring of vascular bundles, inside which are two medullary bundles situated opposite one another.

2. The vascular ring is made up of the leaf-trace bundles.   An arch of bundles entering the stem from each leaf runs perpendicularly through two internodes.   At the end of the second internode it curves inwards and bifurcates.   The two bifurcations fuse with the other two from the opposite side, and give rise to two medullary bundles of the internode below.

3. Some bundles of the leaf-trace, in addition to those which go to form the normal ring, penetrate inwards, and each division joins each of the newly formed medullary bundles.

4. The two medullary bundles bifurcate at the end of an internode, and the four bifurcations join with the four bifurcations of the normal ring. The medullary bundles of the branches behave similarly.

5. At the nodes the vascular rings of the branches, like that of the stem, curve inwards from two opposite sides, bifurcate, and the four bifurcations join with the two bifurcations of the vascular ring of the stem.

6. At the base of the stem the medullary bundles fuse together and terminate in the woody cylinder of the root.

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[1] No reference was found in Link's original work as quoted by de Bary in his book, on p. 590.

EXPLANATION OF THE FIGURES.

All figures illustrating this paper are diagrammatic. The dotted areas show the medullary bundles, the black areas represent the normal vascular rings, and the lined regions indicate the fresh bundles entering the stem from the leaves at the node.

In all figures :

$E.$ = epidermis; $C.$ = cortex; $V.$ = vascular ring.

L_1, L_2, L'_1, L'_2, L''_1, L''_2 = lateral portions of the rings of the stem and two branches.

M_1, M_2, M'_1, M'_2, M''_1, M''_2 = medullary bundles of the stem and branches.

$L.T.$ = leaf-trace bundles.

T_1, T_2, T_3, T_4 = bifurcations of the leaf-trace bundles.

a_1 and a_2	= bifurcations of L_1.		a_3 and a_4	= bifurcations of L_2.	
a'_1 and a'_2	=	,, ,, L'_1.	a'_3 and a'_4	= ,,	,, L'_2.
a''_1 and a''_2	=	,, ,, L''_1.	a''_3 and a''_4	= ,,	,, L''_2.
b_1 and b_2	=	,, ,, M_1.	b_3 and b_4	= ,,	,, M_2.
b'_1 and b'_2	=	,, ,, M'_1.	b'_3 and b'_4	= ,.	,, M'_2.
b''_1 and b''_2	=	,, ,. M''_1.	b''_3 and b''_4	= ,,	,, M''_2.

a_1, a_3, b_1, b_3, a'_1, a'_3, b'_1, b'_3, a''_1. a''_3, b''_1, b''_3 ; T_1 and T_3 give rise to M_3, one of the two medullary bundles of the next internode

a_2, a_4, b_2, b_4, a'_2, a'_4, b'_2, b'_4, a''_2, a''_4, b''_2, b''_4 ; T_2 and T_4 give rise to M_4, the second medullary bundle of the next internode.

Some Observations on the Action of Radium on Certain Plant Cells.

BY

MAUD WILLIAMS.

With three Figures in the Text.

CONTENTS.

I. INTRODUCTION.

IN studying the various changes which may be produced in the complex colloidal system of a living cell it seems desirable to have some method of dealing with the effect of electric charges of one kind only, unhampered by undissociated molecules and ions of opposite charge. The application of radium in suitable fashion may allow one to submit the living protoplasm to this treatment.

Radium and the radium salts are known to emit a-rays (positively

charged particles), β-rays (negatively charged particles), and γ-rays which are similar to X-rays, but distinguished by a shortness of wave-length not yet attained with the most powerful X-ray apparatus. This shortness of wave-length means that the rays are peculiarly penetrating or hard.

There is the possibility that biological changes may be produced by any or all of the three kinds of radiations. Further complications may be anticipated from the fact that secondary radiations can be produced by rays of all three types impinging on matter. Recent continental work (1), however, shows that, as measured by their bactericidal power, these secondary rays are only very feebly effective, and in the work described in this paper no attempt has been made to differentiate between the effects of the primary and the secondary radiations.

In practice the question is considerably simplified by the fact that the α-particles are very readily absorbed by a thin sheet of mica, leaving us with the β- and γ-rays as possible factors.

Although the β-rays are far more penetrating than the α-rays, and differ among themselves in their power of penetration, Rutherford has estimated that the hardest are completely absorbed by 2 mm. of lead. It is therefore possible, by careful screening, to cut off both α- and β-rays and then to study the given cells under the γ-radiation alone. By a comparison of the series of changes produced in the cells by β and γ acting together, and by γ alone, it should be possible to judge whether the β-rays are responsible for any changes it is not possible to produce by the use of γ-rays alone. It has already been established that β-rays have certain effects on cells, because Abbe has deflected them by means of a magnet and used them in treatment of skin diseases without the γ-rays.

If the series of changes in the cells prove to be the same under the two methods of irradiation, then quantitative information must be sought as to the intensity of each type which is needed to produce some definite measure of cell change.

Work on the various rays not only promises information as to the effect of bombarding living cells with negative charges moving with enormous velocities, estimated at 10^{10} to 2.99^{10} cm. per second, and having affinity with the electrons produced when light of a short wave-length falls on matter, but it also affords a comparison between the effects of the ordinary X-rays and the shorter γ type yielded by radio-active bodies, a point of interest now that wave-length relationships are of such importance in chemical and physiological theories.

The account given in this paper is only a preliminary one, and the experiments have only advanced to the point of dealing with material under conditions such that both β- and γ-rays act upon the cells. It has been interesting to find how greatly the effects described below resemble those produced in the same material by the action of X-rays (8). When it is

remembered what great importance is attached to the action of radium upon cells of malignant growths at the present time, it seems well worth making a close investigation of its action upon living cells, easy to obtain and observe in plant tissues.

II. *Résumé* of Work of Earlier Workers on Living Cells.

a. Results obtained on plant material.

In the experiments described in the literature dealing with the action of radium upon plant tissues we find investigations have been attempted mainly from three points of view:

(*a*) Lethal effects on bacteria and other lowly forms.

(*b*) Influence upon the growth-rate and ultimate form of the individual.

(*c*) Influence upon respiration and photosynthesis.

In many of the earlier papers the exact quantity of radium salt, and often even the chemical nature of that salt, is not known, and frequently the method of presentation is not mentioned; so that it is not possible to obtain any connexion between intensity of radiation and the effect.

Experiments upon emulsions of *Bacillus prodigiosus, Streptococcus*, &c., were made by Chambers and Russ (2), who exposed the preparations to radium. The specimens were found to be sterilized in times of exposure which depended upon the dose of rays used. At a later date Packard (3) experimented upon *Spirogyra* treated with 20·4 milligrams of radium bromide kept in a silver tube in the water in a watch-glass with the alga. He described the changes as follows: ' The chlorophyll bands first contract somewhat, losing their spiral arrangement. A few minutes later they resolve into an irregular heap of chlorophyll in one end of the cell.' The important point brought out in this work was the fact that the shrinkage was produced less quickly in the light than in the darkness. This writer also made experiments upon protozoa and found organisms possessing chlorophyll to be more resistant than those without.

As regards the second group of experiments there seems to be discordance in results, possibly due to lack of knowledge of the quantities of radio-active material used. Thus Molisch in 1912 found he could accelerate the opening of buds by treatment with radium, while in 1916 Brick (4), who used both rays and emanation (the gas produced during the transformation of radium, which in its process of decay forms products yielding a- and β-rays), found the effects were always harmful. Growth of both root and shoot was retarded, starch formation was inhibited, and curious formative effects were noted.

More recently the germination of radish seed under the influence of radium emanation has been studied by Redfield and Bright (5).

In these experiments the seeds were packed round the tube containing the emanation and were kept at constant temperature. Unfortunately no information is available about the quantity of radiation. The germination was retarded and no relation was found between the CO_2 production and the power of germination.

Experiments upon germination, growth, and metabolism are especially interesting because there is growing evidence that enzymes can be affected by radiations. In fact, in the hands of Hussey and Thompson (6), experiments upon trypsin have advanced to the stage of showing that the decomposition produced by emanation varies with the concentration of the trypsin preparation, the quantity of emanation, and the time of exposure.

In the third group of experiments attempts were made by Hebert and Kling to replace sunlight by radium emanation in work on photosynthesis (10). No success was reported, but the emanation was not without its influence upon the cells, because after exposure ended both respiration and photosynthesis were reduced.

b. Results obtained on certain animal tissues.

A much greater amount of work has been done upon the animal side. The effects of irradiation upon animals, as shown in subsequent development, have been studied, and detailed work has been carried out upon the tissues of malignant growths; both upon the structural changes in the cells and upon their powers of growth when portions are grafted into healthy animals. This work is far too lengthy and complicated for discussion here, but certain features are particularly interesting, and it will be seen later that there is close agreement between some of the changes it is possible to produce in plant and in animal tissues.

Forsdike (7), in summarizing recent work, states that radium rays show a differential action upon animal cells of various kinds, and that this differential effect is more marked in rapidly growing and dividing tissues than in mature cells. It is also found that while 'strong' doses may procure effects in a few hours, with 'moderate' doses there is a definite latent period before any change is noted, 'but the microscopical changes are identical, though delayed'.

In the changes described in the literature there are many cases of profound alteration of the character of the cells as seen in comparison with controls similarly stained. Great vacuolation is a marked feature, and in some cases long exposure leads to almost total disappearance of cytoplasm, the remnants being granular in structure.

The interpretation of the changes produced in animal cells is of great difficulty because the observations are often made upon organs still intact within the body during the irradiation, and it is impossible to say what part

of the results is due to direct action upon the cells, and what part arises from disturbances in the blood leading to secondary action in the tissues.

On considering the subject from various aspects it appears that treatment of certain plant cells, such as those in the petioles of *Saxifraga umbrosa*, is likely to be a profitable way of studying the series of changes which leads up to the final and irreversible state of injury which so many workers have shown may be the outcome of the irradiation of living matter. The material has the following advantages:

1. Large quantities, grown under similar conditions, can be obtained.

2. Cells in the same mature condition and of the same type can be studied.

3. The material can be examined in the living condition at various stages of treatment, and the experiments are not vitiated by methods of fixing and staining.

4. The material, being non-motile, can be exposed to constant intensity of radiation throughout the test.

III. METHODS EMPLOYED.

For the tests detailed below, strips of tissue, torn from the upper surface of the petioles of *Saxifraga umbrosa*, formed the material chiefly used. Such strips, consisting of the epidermal layer together with the adjacent layers, offer cells of rectangular form, of great uniformity. The tissue is particularly rich in tannin, and a weak solution of $K_2Cr_2O_7$. applied to the cells, had already been found a useful indicator of an irreversible state of injury. Methods of growth of material and of preliminary washing have been described previously (8).

Two different supplies of radium salts were used at various times. In the earlier tests use was made of a large supply (reputed of the order of 0.1 grm.), for the loan of which the writer is indebted to the late Sir A. Reid. This salt was not standardized, and was enclosed in a platinum tube of unknown thickness. It was found by experiment that cells were changed equally anywhere over a length of 1.5 cm. in the middle of the tube. When working with this amount of a radio-active material precautions are needed, and, in order to present the sections to the action without risk to those in the neighbourhood, a lead cradle was made to hold the tube. An aperture was provided in the upper and lower surfaces, so that slides could be exposed parallel to the length of the tube. When it was desired to alter the intensity of radiation used, the cradle was supported horizontally above the bench, and the slides were fixed across a series of rings adjusted to measured distances above and below the tube.

It was necessary to remove the slides when examination of sections by the microscope or ultra-microscope was carried out. All the tests had to

be limited to those at the temperature of the room. By these means the series of changes was studied, and in quoting the results later the radium will be called ' radium A '.

The second source was a small circular capsule weighing 6·4 grm., and provided with a thin mica window, of 0·9 cm. diameter, on the upper side. This capsule was tested at the National Physical Laboratory, and reported as ' 0·51 mg. of hydrated radium bromide, of effective γ-ray activity equivalent to 0·27 mg. radium element in radio-active equilibrium '. When this source, quoted later as ' radium B ', was used, it was possible to arrange sections on a warm stage on the microscope, raise the objective, and lower the capsule, mica downwards, on the cover-slip over a chosen portion for the time desired. The effects of the pressure due to such a capsule were, of course, tested separately. With this arrangement there were the advantages of continuous observation on the same cells and of temperature control.

With radium A the exposures were made mainly in the diffuse light of the laboratory, but with radium B sets of tests were made respectively with the microscope mirror brightly illuminated during exposures, illuminated by the diffuse light of the room, and darkened. In all experiments cover-slips of large size, which had been tested with a spherometer for uniform thickness, were used.

The material for treatment was mounted in distilled water in the earlier tests, and in tap water in the later ones. No differences could be detected due to the difference in liquid.

In all cases where effects were tested twenty-four hours after irradiation the specimens were immediately transferred to beakers of fresh tap water, and were kept in darkness at the same temperature as the controls. By this change of water it was sought to eliminate secondary effects which might be due to the chemical changes some workers have suspected take place in water itself after long action of X-rays and radium.

It will be seen that in all the tests made the material has been submitted to the joint action of β-rays and γ-rays, for the methods of enclosing the radium prevented α-rays reaching the cells.

When radium A was used the β activity was of course diminished by the platinum case, but lack of information about the thickness of metal prevents an estimate of the actual value received by the cells. The present account can only be regarded as a preliminary record, and has been written only because some of the qualitative aspects seem of interest.

IV. Description of the Changes seen in *Saxifraga umbrosa*.

a. General course of events.

The general course of events was observed under radium A. The first change was a slight increase of circulatory rate (measured as already described for X-ray experiments) (8), and, after a short period of further

treatment, there was a noticeable increase of the amplitude of the Brownian movement of minute particles within the cells. Now if microscopic particles of constant size and character show an increase in the amplitude of their Brownian movement, while temperature conditions, &c., remain the same, it can be taken as an indication that the viscosity of the medium in which they move has diminished. This suggestion of a lowering of viscosity has also been found when the same plant material was treated with X-rays and the time of exposure was short. The results at first sight appear to be in opposition to those of Weber (9), but the times allowed for irradiation, and the period which seems to have elapsed between treatment and the testing of the viscosity by his method of centrifugalizing, may well account for the differences. In the cells of *Saxifraga* the Brownian movement died down as the time of treatment was increased.

The increase in circulatory rate also proved only temporary and began to fall off with longer exposure, and then changes in the nature of the protoplasm became visible. Just as with X-rays, there was a gradual shrinkage of cytoplasm from the cell-wall, although at first a smooth contour was maintained. Later irregularities appeared and the mass became granular, while with dark-ground illumination there was more scattering of light than in the case of controls. Finally, a stage was reached when the sections showed the entrance of $K_2Cr_2O_7$ into the cytoplasm and a yellowish precipitate within the time-limit chosen (three minutes).

Although this change in the cells was quite general and the precipitation clear, there was not that depth of coloration in the given time that is found when these tannin-containing cells have been rendered permeable by immersion in electrolytes. That there is a suggestion of a definite relationship between the intensity used and the time of action needed to produce this permeability condition will be seen later.

Exposure prolonged still further produced great changes in the appearance of the cells. In some, tiny dense masses of granular nature lay in the centre, in others there were signs of enormous vacuolation, and in all cases the protoplasm became discoloured. No attempt was made to examine the cells except in the whole state. The cellulose wall remained quite clear and was apparently unchanged.

When this series of changes had been observed, as this large supply of radium was no longer available, attention was turned to the action of the small but standardized amount.

It was soon found that, although the end-effect could be produced, the time needed made experiments upon intensity and time relationships impracticable. It was, however, possible to make interesting tests on the influence of minute doses upon circulatory rate, and on certain aspects of permeability.

b. Circulatory rate.

A series of exposures was made at different dates in the year to find whether quantities as small as radium B could stimulate the circulatory rate. The actual exposures of the sections of *Saxifraga* were made in darkness, for we have seen there is reason to expect the maximum action in such conditions.

Specimens and their controls were maintained throughout at room temperature, and care was taken to keep all focusing arrangements constant. Since light itself may have an influence upon circulation, all material

TABLE I.   *Circulatory Rate under Radium Treatment.*

Material: Strips from the petiole of *Saxifraga umbrosa*.   Temperature: Room temperature.
  Times for movement over twenty scale divisions taken.   Sixteen readings averaged for each
  value.   Cover-slip thickness, 0·035 cm.

Date. 1922	Time of Exposure.	Readings immediately after Exposure.		Readings 24 hrs. after Exposure.	
		Control.	Specimen.	Control.	Specimen.
Feb. 22	25 min.	33·1 sec.	20·5 sec.	23·6 sec.	27·6 sec.
Feb. 22	25 min.	29·4 sec.	22·6 sec.	21·7 sec.	33·7 sec.
March 1	35 min.	12·5 sec	12·2 sec.	12·2 sec.	17·9 sec.
Nov. 1	50 min.	10·9 sec.	10·7 sec.	11·2 sec.	12·5 sec.
Nov 6	40 min.	15·5 sec.	13·2 sec.	12·1 sec.	14·9 sec.
Nov. 8	45 min.	13·9 sec.	16·5 sec.	11·4 sec.	13·5 sec.

was left for ten minutes under the microscope before readings were begun. The times needed for the bright particles to move over twenty scale divisions were then observed. Sixteen readings, taken in quick succession, over different cells were averaged to give each of the values quoted. Table I will suffice to show that the stimulatory effect of short exposure to a quantity of this order is general. The readings taken twenty-four hours after treatment show that in all cases a reaction has occurred leading to a diminished rate in comparison with the controls. As this alteration of circulatory rate was an interesting point, it was decided to try to confirm the above impressions by observations on the movement of chloroplastids in the leaf of *Elodea*. In these tests a given cell was focused, and a series of readings of the rate of movement over a special part of the scale was taken at different stages of irradiation. The illumination was kept constant. The initial rate was taken as unity, and rate of circulation and time of exposure curves were plotted. Here again the influence of the pressure exerted by the capsule holding the radium had to be investigated. It proved to be greater than in the case of the *Saxifraga*, but still of small importance. Graphs showing representative cases of these fluctuations under pressures are given in Figs. I *a* and I *b*. The cover-slip was weighted in such a way, that the sections were submitted to the same pressure as that caused by the capsule. Some representative changes under radium are plotted in Figs. II *a*

and II *b*.　It will be seen that the changes are of much greater range, and there is early stimulation followed by depression under longer exposure.
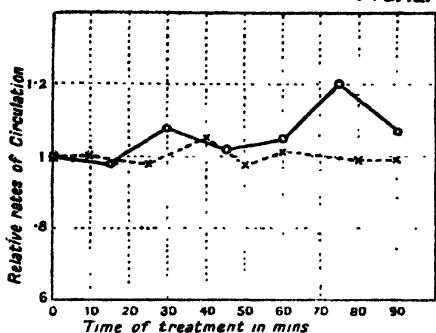
FIG. Ia.　　　　　　　　　　　　　　　　**FIG. Ib.**
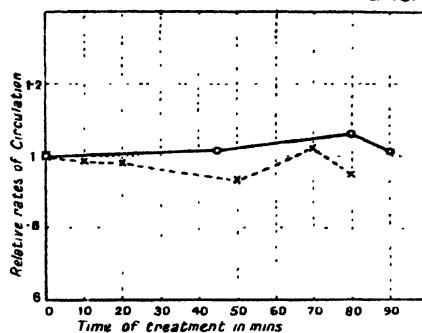
FIGS. I *a* and I *b*.　Circulatory rate in *Elodea* and effect of pressure.

	Date.	Time Pressure was applied.	Temperature of Warm Stage.
1	March 1, 1923	10 min.	15.8°–16.2° C.
2	March 8　,,	15 min.	19° C.
3	Feb. 28　,,	10 min.	21° C.
4	March 7　.,	45 min.	15.6° C.
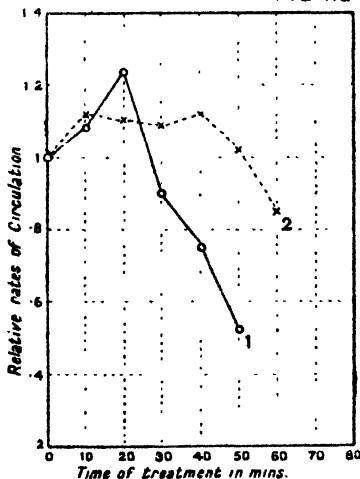
FIG IIa　　　　　　　　　　　　　　　　**FIG. IIb.**
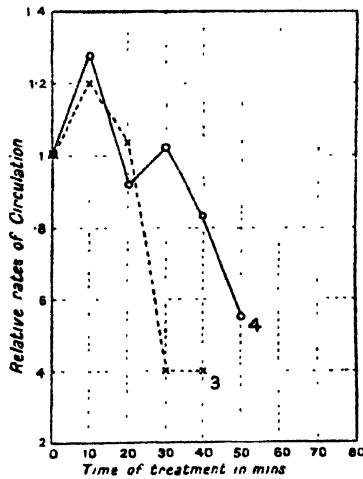
FIGS. II *a* and II *b*.　Circulatory rate in *Elodea* under radium treatment.

	Date.	Temperature of Warm Stage.
1	Dec. 13, 1922	19° C.
2	Dec. 18,　,,	17.6° C.
3	Dec. 7,　,,	20° C.
4	April 25, 1923	16° C.

In all, eighteen experiments were carried out on *Elodea* at various dates between November 1922 and October 1923.　Of these, fifteen showed

slight acceleration under treatment for periods as short as ten to twenty minutes, while fourteen showed depression of the initial rate before the first hour of treatment was ended.   The results obtained for *Saxifraga* were therefore confirmed, but the *Elodea* appeared more sensitive to the treatment, especially when it is remembered that the exposures took place in the light, when sensitivity is probably not as great as in darkness.

c. Stomatal aperture.

As in the case of the experiments with X-rays already mentioned, readings of the average stomatal gap (ratio of the short axis to the long) were made to find whether there was any suggestion of loss of turgor before the stage was reached when the cells gave the reaction with the $K_2Cr_2O_7$. As in these tests the pressure of the capsule might conceivably alter the turgor of the cells, preliminary experiments upon the pressure factor were needed.   For all these stomatal gap experiments strips from the under-surface of the leaf-blade were used.   All were left in water for half an hour before use, and all sections were left under the microscope for another period of ten minutes before readings began.   Table II *a* shows that the differences between the strips under mere pressure and their controls fall within the limit of experimental error, while Table II *b* shows that the influence of the radium is to produce a diminution of the ratio, which implies a loss of turgor.   Since the material is completely immersed in water during the experiments, this change of turgor can only be attributed to disappearance of substances possessing osmotic properties from the cell-sap. Now there has never been any suggestion put forward by workers on these subjects that the action of radium is to cause the production of more complicated compounds which would lessen the osmotic pressure, so that the only explanation which can be given is that the rays make the protoplasm more permeable, and that solutes escape from the cell in consequence.   The lowering of the osmotic pressure would then lead to a reduction of the width of the stomatal pore.   The suggestion of exosmosis of material from the cells, so diminishing the turgor, has been confirmed by measurements of the stomates in *Tradescantia* leaves, and by watching the gradual loss of colour from the purple cells of this plant when under the action of the rays. There has been a theory put forward by some medical writers that the changes they have produced could be accounted for by a decomposition of complex substances leading to a rise of osmotic pressure and consequent influx of water during early stages of action.   It was thought that readings of the stomatal gap, after very short periods of exposure, might show an increase in the ratio and confirm the idea of a rise of osmotic pressure, but no evidence in support of this has been found in the present work.

TABLE II. *Stomatal Gap in Saxifraga umbrosa.*

A. Effect of pressure equal to that caused by radium capsule.

Conditions: Temperature constant. Sections in light of microscope mirror for 10 min. before readings taken.

Date.	Temp.	Average Value for Stomatal Gap.		% Change.
		Specimen.	Control.	Probable
1923				Error 3 %
Nov. 28	17° C.	0·44	0·44	0
Dec. 4	19° C.	0·44	0·43	+ 2·3
Dec. 5	19° C.	0·49	0·48	+ 2
Dec. 11	19° C.	0·42	0·42	0
Dec. 12	19° C.	0·49	0·50	− 2
Dec. 18	20° C.	0·43	0·45	− 4·4

B. Radium effect through cover-slip of 0·012 cm. thickness.

Date.	Temp.	Conditions of Exposure.	Value in Specimen.	Value in Control.	% Change.
1923.					
Oct. 24	19° C.	1·75 hrs. in darkness	0·42	0·51	− 17·6
Oct. 30	19° C.	20 min. in darkness	0·44	0·53	− 17
Oct. 31	19° C.	25 min. in light	0·41	0·47	− 12·8
Nov. 6	16·5° C.	15 min. in light	0·48	0·46	+ 4·3
Nov. 13	17° C.	35 min. in light	0·36	0·45	− 20
Nov. 14	20° C.	20 min. in light	0·38	0·43	− 11·6
Dec. 12	19° C.	30 min. in darkness	0·45	0·50	− 10
Dec. 18	20° C.	40 min. in light	0·39	0·45	− 13·3

d. Plasmolysis experiments.

An endeavour was next made to find whether any difference of osmotic pressure could be actually detected in the living cells after exposure to the radium, and before visible changes in the state of aggregation and vacuolation of the protoplasm had been induced. It was realized that any determinations would have to be taken very quickly after exposure ceased, in order to avoid after-effects which will be mentioned in a later section. For this reason it was not practicable to find the concentration of applied solution which would produce visible shrinkage in a fixed percentage of cells in a given time, and then to correct for wall-extension factors. All that this type of work would allow was a preliminary rough determination, by the plasmolytic method, of the osmotic pressure of leaves in the same region of the plant as the irradiated specimens, and then an estimation of the percentages of cells in control and specimen which could be visibly changed by ten minutes' immersion in the lowest concentration of sucrose found to be effective in the preliminary tests. The first step was to examine plant material to find cases where adjacent leaves gave the same values for the osmotic pressure. *Elodea* was soon ruled out because not only did neighbouring leaves show great differences, but the cells of the same leaf differed among themselves. The upper epidermal strips of the *Saxifraga* were

found to be suitable if regions in the middle of the petiole were used ; these allowed examination of 100 to 200 cells.   Table III A shows the very close agreement in percentages of the plasmolysed cells in two adjacent leaves. It will be noticed there were wide fluctuations in the concentrations of applied sucrose needed to show shrinkage in the given time-limit in rosettes cut at various dates.

TABLE III A.   *Study of Osmotic Pressure Variation in Central Regions of Epidermal Strips from Adjacent Leaves of Saxifraga.*

Ten minutes' immersion.

Date.	Lowest Concentration producing Effect in the Time.	Percentage of Cells changed in Series of Leaves.
1924		
March 26	0·3 grm. mol. sucrose	8, 6
March 11	0·21 grm. mol. sucrose	13, 12
March 11	0·21 grm. mol. sucrose	10, 9
March 18	0·25 grm. mol. sucrose	4·6, 3·1, 4·4
April 1	0·22 grm. mol. sucrose	7, 5
April 2	0·26 grm. mol. sucrose	4, 6
June 11	0·22 grm. mol. sucrose	10, 15
June 17	0·22 grm. mol. sucrose	14, 17. 16
June 17	0·22 grm. mol. sucrose	2, 7, 4
June 18	0·22 grm. mol. sucrose	10, 10

In Table III B are seen the results for irradiated specimens and the controls ; it is evident that plasmolysis is more readily induced in the cells which have been under treatment.   In no case was there any evidence for an increase of osmotic pressure.   Thus both the stomatal pore experiments and the plasmolytic tests point to a decrease in the water-attracting power of the sap, which it seems only possible to explain on the assumption of permeability change.   It seems significant that on a few occasions after long exposure there was only a transitory plasmolysis, and the sucrose must then have been able to enter the cells.

TABLE III B.   *Influence of Radiations on Plasmolysis.*

Date.	Tempera- ture.	Time of Radia- tion.	Grm. mol. conc. of Sucrose applied.	% of Cells changed in Control.	% of Cells changed in Specimen.
1924					
March 11	17·5° C.	1 hour	0·21	11	34
March 15	17° C.	1·75 hours	0·21	6	14
March 18	16·5° C.	1 hour	0·25	4	15
April 2	15·5° C.	1·25 hours	0·26	5	12
May 6	16·5° C.	1 hour	0·22	5	33
May 7	15° C.	1 hour	0·22	21	60
May 27	18° C.	1 hour	0·22	0	12
May 28	19° C.	1·33 hours	0·22	1	20
June 18	25·5° C.	1·5 hours	0·22	7	28

V. Certain After-effects.

In addition to the depression of circulatory rate shown to follow small doses of the radiation, there were after-effects of more profound nature which followed longer treatment.

If the radiation ceased before any shrinkage of protoplasm had taken place and the material were then placed in fresh tap water and kept in darkness for twenty-four hours or longer, great differences between its cells and those of the control became visible. The protoplasm, often very discoloured, either collected in a dense mass in the middle of the cell or became very dense at one end and very vacuolated at the other. The chloroplastids did not lose their characteristic colour or appearance. Where a long strip of *Saxifraga* was irradiated, so that the radium only acted upon the middle strip portion, there was a striking difference between the cells that had received radiations and those beyond the aperture of the capsule.

The length of exposure needed to produce this appearance as an after-effect depended upon whether *Elodea* or *Saxifraga* was used, and also upon the time of year when the tests were made.

All this made it plain that any attempt at time and intensity relationships must be made at one fixed season of the year, and tests carried out immediately irradiation ceased.

There is apparently nothing known as to the type of change which produces these after-effects, although the literature contains many records which show that such must occur. It will be sufficient to refer to the quotation from Forsdike and to the findings of Hebert and Kling already mentioned.

If suitable supplies of radium become available it is hoped to carry out some tests on the relationship between the latent time and the exposure given, and also to study how the values are affected by temperature. As a minimum exposure of three hours to radium B was needed to produce the after-effects described, numerical work on these subjects was not feasible, with this small quantity.

VI. Preliminary Attempt at Time and Intensity Relationships.

In the summer of 1920 a preliminary piece of work was done with radium A upon the times of treatment needed for various intensities of radiation (β- and γ-rays) to produce the permeability to $K_2Cr_2O_7$. The cells were all in a mature state, and for the few tests made as late as November the plants were kept in a greenhouse. Although only a few readings were possible it was seen that the time needed clearly depended upon the intensity

applied. The readings are recorded in Table IV, while Fig. III shows the variation of the time with the distance of the radium. For the arrangement

FIG. III.
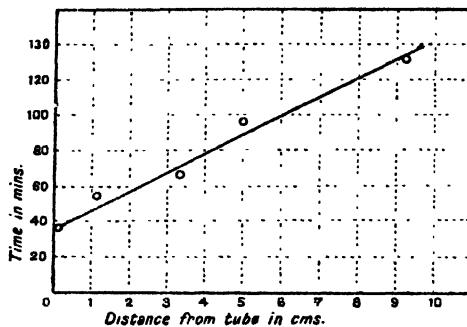
FIG. III. Relation between time of treatment needed to produce 'end-effect' and distance of radium source from specimen.

used it may be assumed the intensities vary inversely as the squares of the distances of the slide from the radium tube when the latter is placed horizontally in the lead cradle, and the slide is parallel to the gap.

TABLE IV.

T, time of exposure to produce permeability to given $K_2Cr_2O_7$. D, length of air-gap between platinum tube and cover-slip. Cover-slip 0·016 cm. thickness approximately. Experiments at room temperature in diffuse light.

Date of Expt.	D.	T.	Average T.
1920			
June 28	0·1 cm.	35 min.	
June 30		30 min.	
June 30		35 min.	36 min.
July 1		40 min.	
July 1		40 min.	
Sept. 29	1·1 cm.	50 min.	
Sept. 30		55 min.	
Oct. 2		57 min.	54 min.
Oct. 2		57 min.	
July 9	3·3 cm.	65 min.	
July 12		60 min.	
Sept. 24		65 min.	65 min.
Sept. 29		70 min.	
Nov. 24	5 cm.	95 min.	
Nov. 24		95 min.	
Nov. 25		100 min.	97 min.
Nov. 25		100 min.	
July 12	9·2 cm.	120 min.	
July 16		130 min.	
July 19		135 min.	130 min.
Sept. 23		135 min.	

SUMMARY.

1. Changes produced in the cells of *Saxifraga umbrosa* and of *Elodea* under the combined influences of β-rays and γ-rays from radium are described.

2. The first effect noted under short exposure is an increase in the rate of circulation.

3. In the early stages of irradiation there is reason to suppose that the viscosity of the protoplasm is lowered, but only temporarily.

4. Experiments are described which show that the cells are rendered permeable and slow exosmosis of solutes can occur before any visible changes are found in the protoplasm.

5. No evidence of an increase in the osmotic pressure in the early stages, such as might have been expected from some of the medical literature on the subject, has been found.

6. Large dosage produces shrinkage, and vacuolation effects, similar to those described by workers on animal cells.

7. There is evidence of important after-effects.

8. There is every indication that at various seasons the plant material varies in its vulnerability.

9. For experiments carried out with a large quantity of radium there appears to be a definite relationship between the intensities used to produce a certain end-effect and the times of action needed.

10. One cannot but be struck by the close similarity of the changes induced in *Saxifraga* by the joint action of the β-rays and γ-rays of radium, and those caused by the softer rays of the γ type known as X-rays. No change has been seen under the combined actions of the negatively charged particles and the etheric radiations which has not already been found as a result of the radiations alone (8). One cannot hope to elucidate the part which may possibly be played by β-rays in stimulation and coagulation until quantitative work on γ-rays is available. Experiments in this direction are now in progress.

11. From the time any change is seen in the appearance of the protoplasm in all cases that change is irreversible.

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Carboniferous Bryophyta.

I. Hepaticae.[1]

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With Plate XIII and one Figure in the Text.

OUR knowledge of the geological history of the Bryophyta is very meagre, a fact not altogether unexpected in view of the relatively fragile nature of the plant-body in that group of organisms. So far as concerns the Palaeozoic the records are few, and beyond indicating the existence of the group in that epoch tell us practically nothing about their structure or relation to their modern representatives. When we restrict our discussion to a consideration of the Hepaticae, one of the two main divisions of the Bryophyta, we find that there is no record in Palaeozoic rocks of a plant that can with any certainty be attributed to that division. Raciborski has described several [2] thalloid organisms (*Palaeohepatica* spp.), some from rocks as early as the Trias, but according to subsequent writers [3] these organisms are quite as likely to be Algae, and do not afford any reliable information about the Hepaticae. The application of the term *Marchantites* to thalloid plants of admittedly unknown affinities has led to considerable misunderstanding. Pelourde [4] cites Kidston's [5] record of *Marchantites* in Lower Carboniferous Rocks of Scotland as a demonstration of the existence of Hepaticae during that period. Dr. Kidston has, however, informed me that he was employing the term *Marchantites* in a wider sense and not intending to imply the presence of plants related to the Bryophyta. He latterly suggested the use of the word *Thallites*

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[1] The occurrence of the Carboniferous Liverworts to be described below was recorded by the writer in the Proc. Manch. Lit. and Phil. Soc., Nov. 1924.

[2] Raciborski (1894) : Flora Kopalna, Pamiet Akad. Umiejetnosci.

[3] Potonié und Gothan (1921) : Lehrbuch der Paläobotanik, p. 35.

[4] Pelourde (1914) : Paléont. végétale, p. 21, Paris.

[5] Kidston (1901) : Summary of Prog. Geol. Surv. U.K. for 1900.

for such indeterminable thalloid fossils. Carrying out Dr. Kidston's suggestion we may define the form-name *Thallites* as follows :

Fossils in which the plant body is of a thalloid form, as may be found in the Algae, Bryophyta, or sometimes in higher groups; but possessing no characters by which they may be assigned to any one of these groups to the exclusion of all the others.

This form-name is applicable to all fossils which up till now have been named *Marchantites*, except those such as *M. Sezannensis*, Sap.,[1] which bear undoubted affinities to the Marchantiaceae. Thus *Marchantites* (*Fucoides*) *erectus*, Leck.,[2] should, in accordance with this new nomenclature, be termed *Thallites erectus*, Leck., for according to Professor Seward[3] it would be ' unwise to speak with any confidence as to the real affinities of the fossil '. Probably most of the fossils of a thalloid nature termed *Fucoides* should also be given the form-name *Thallites*.

Dr. L. Wills describes a ' thalloid growth ' from shales of Upper Coal-Measure Age in Staffordshire which she suggests may be a Liverwort.[4] The evidence from this plant alone is not strong, but I shall bring forward strong confirmative evidence for regarding this fossil as one of the Hepaticae. It would therefore appear to be the first recorded evidence we have of the occurrence of Liverworts in the Palaeozoic.

It is to be remarked that records of Mesozoic Bryophyta are as scarce as those of the Palaeozoic, and that not until we deal with literature on Tertiary plants do we encounter many references to fossil Bryophyta.

While examining some samples of shales from the Etruria Marl Group at Old Hill in Staffordshire, I discovered some specimens of a thalloid organism which I later identified as the plant described by Dr. Wills. In addition to giving me information about the provenance of the specimens she had described, Dr. Wills very generously sent me all the preparations she had made of this interesting plant. Her brother, Dr. L. J. Wills of Birmingham, has very kindly supplied me with some fine-grained shales of Middle Coal-Measure Age from Shropshire, from which I have been able to extract some fossils which are undoubted Liverworts. In view of the discovery of some hitherto unnoticed characters of the thalloid plant, I propose to refigure it and add a few details to our knowledge of its structure.

The specimens were isolated from the shale by treating it with hydrofluoric acid, which was found to be the most satisfactory reagent. It was found that by mounting the pieces of shale on a slide which was later coated with wax, additional ease was acquired in manipulation. The

[1] Saporta (1868) : Mém. Soc. Géol. France, vol. viii, p. 308.

[2] Leckenby (1864): Quart. Journ. Geol. Soc., vol. xx, p. 74, Pl. I, 2 A, 2 B.

[3] Seward (1898) : loc. cit., p. 234.

[4] Wills, L. (1914) : Geol. Mag., N.S. Dec. vi, vol. i, No. 9, p. 388, Pl. XXXI, Fig. 8.

same method of mounting that is employed in making a transfer preparation[1] can be used. The sample mounted in this manner was examined from time to time during the hydrofluoric treatment, and organic remains that became loosened were removed and then mounted for examination under the microscope. Another advantage of this procedure is that the shale does not crack up into large fragments as it does when an unmounted piece is put into the acid direct, but is slowly converted into a fine mud at the surface exposed to the action of the acid. The plant fragments emerge therefore in a comparatively undamaged state.

DESCRIPTION OF THE SPECIMENS.

The name *Palaeohepatica*, Raćiborski, was instituted for fossil plants of thalloid habit, but which cannot with certainty be said to be Liverworts ; it is therefore inapplicable to the plants which will be described below. On the other hand Saporta's term *Jungermannites* implies a definite group relationship which under the present circumstances is to be avoided. To settle these difficulties the name *Hepaticites* is suggested ; it is defined as follows :

Hepaticites, gen. nov.

Fossil plants bearing evidence of a relationship to the living Hepaticae, and also exhibiting characters by which they may be distinguished from the Algae, Pteridophyta, and other divisions of the Plant Kingdom, are to be assigned to the form-genus *Hepaticites*, if the knowledge of their structure is too incomplete to warrant the use of a distinctive generic term.

Hepaticites Kidstoni, sp. nov.[2]

While engaged in making a preparation of some specimens of a *Lepidophyllum*, with the intention of studying the cuticular structure, I found the specimen figured on Pl. XIII, Figs. 1 and 2. It was so obviously a Liverwort, and modern in appearance, that at first I was not satisfied that it actually came from the shale, and was not an impurity which had been admitted in the tap water used to wash the preparation. Fortunately, with carefully selected unfissured pieces of shale, and employing pure reagents, I obtained another specimen, and, what was still more convincing, saw this specimen in the condition of being only half freed from the shale. There is then no doubt as to its true origin in the shale, which in addition contains *Lepidodendron* and other plants. The specimens so obtained were washed, dehydrated, cleared, and mounted in balsam. They are all very

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[1] Walton, J. (1923): this Journal, vol. xxxvii, No. cxlvii, July, 1923.
[2] The diagnoses of the species described will be given in Part II.

fragile and break up exceedingly easily ; the most perfect fragment (Pl. XIII, Fig. 1) has, since being mounted and photographed, split into several pieces owing to slight strains in the balsam.

It will be realized how small these plants are when one considers the magnification of the plate-figures. *Hepaticites Kidstoni* can be compared in size with the smaller modern foliose Liverworts. The condition of preservation is remarkable, practically every cell being clearly defined. The plants seem to be preserved in a condition much resembling that of herbarium specimens.

The plant is differentiated into axis and leaves. The latter are of two kinds ; there are two rows of large leaves (Pl. XIII, Fig. 1), one on each side of the axis, which is comparatively wide, the members of one row alternating in position with those of the row on the other side. These large leaves are somewhat rhombic in shape ; they vary in size (contrast the top and

a   b
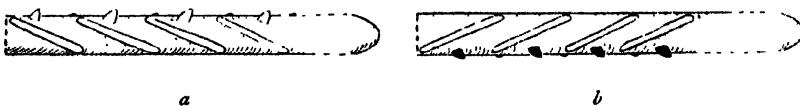
Alternative hypothetical reconstructions of *Hepaticites Kidstoni* as seen in profile.

bottom leaves in Pl. XIII, Fig. 1) and have a distinct set or inclination to the axis of the shoot, which gives an indication of the polarity of the latter, and suggests that in the figures of the specimens (Pl. XIII, Figs. 1 and 2) the anterior end of the shoot is uppermost. This is assumed to be the case in the rest of the description. The posterior and anterior margins of the large leaves converge to a somewhat rounded apex. They are attached to the axial part of the plant by a slightly expanded and decurrent base. The plane of the leaf is nearly parallel to the axis, but as the leaves slightly overlap at their base (Pl. XIII, Fig. 2) they must have been set at an angle to it, and, owing to the compression of the plant and the settling of the matrix during fossilization, the angle of inclination to the axis may have been originally 45° or thereabouts (see Text-fig. above).

In addition to the large laterally spread leaves there is opposite to, and close beside each of them, attached to the surface of the axial portion, a smaller leaf-like structure. As there is one of these structures to each of the large leaves (Pl. XIII, Fig. 4, *l*), they also form two alternating series, one on each side of the axis. As both series of small leaves can be seen to lie on the same surface of the shoot, the latter is definitely dorsiventral. By careful focusing it is possible to determine that the small leaves are only one cell thick. They are in the form of approximately semicircular flaps and are attached by a straight base to the surface of the axial portion (Pl. XIII, Fig. 4, *l*).

If one assumes that the small leaves are situated on the dorsal surface of the shoot, then the latter would have appeared in profile as shown in Text-fig. *a* (note that in Pl. XIII, Fig. 1, the anterior end of the shoot is assumed to be uppermost), and the arrangement of the large leaves would be succubous. Conversely if the small leaves are on the ventral side then the arrangement of the large leaves is incubous (Text-fig. *b*). These positional relations of the leaves can be determined by careful focusing at the places where the latter overlap one another·

The axial region is straight, and the cells are about three times as long as broad (Pl. XIII, Fig. 4). The large leaves consist of a single cellular sheet, the cells of which, in the process of fossilization, have been flattened so that the upper and lower surfaces are now in one plane ; but the outlines of the two free walls of each once solid cell are *out of register* (Pl. XIII, Fig. 3). The cells in the middle of the leaf are considerably larger than those towards the margins (Pl. XIII, Fig. 4). There are several isolated cells with dark contents scattered over the large leaves (Pl. XIII, Figs. 1 and 3, *o*). Waste products may have been aggregated in these cells, but it is also possible that they may have been secretory units. The groups of cells (Pl. XIII, Figs. 1, 2, and 3, *ff*) with dark granular contents probably contained some fungus or saprophytic organism.

There is nothing known about the nature or position of the reproductive organs of this plant.

Locality : Associated with *Lepidodendron* sp., *Neuropteris* sp., &c., in fine-grained argillaceous shale from Preesgweene Colliery, Preesgweene, 2½ miles north of Oswestry, Shropshire.

Horizon : ' Middle Coal Measures ', Yorkian Series, Upper Carboniferous.

Hepaticites lobatus, sp. nov.

This fossil, of the same provenance as *H. Kidstoni*, is similar in texture but is simpler morphologically. There is less distinction between axis and leaves than in the previously described plant. Like the latter it is of small dimensions (Pl. XIII, Fig. 5). The shoot is dorsiventral and is differentiated into a central axial region, possibly as much as three cells in thickness, with an extension of the thallus to form a wing on both sides, the lobes on one side alternating with those on the other. The bays between the lobes may be so deep that they are separated from the axial region by only two or three cells (Pl. XIII, Figs. 5 and 7).

The whole plant consists of parenchymatous tissue : in the axial region the cells are somewhat elongated compared with those in the wings, which are roughly cubical in shape. Scattered over the axial region are groups of cells which are darker in colour than the rest, and some of these, if not all, owe their appearance to the presence of fungi. In another

fragment, probably a part of the same individual, certainly of the same species (Pl. XIII, Fig. 7), there are rhizoids in organic connexion with cells of the axial region (Pl. XIII, Fig. 7, *rh*). In this specimen only the bases of the rhizoids are represented, but in another specimen, which must also be attributed to this species, considerable portions of some of the rhizoids are preserved (Pl. XIII, Fig. 6, *rh, rh*). The rhizoids are in groups which were somewhat sparingly distributed along the axis, as there are two groups separated by a distance of 0·2 mm. (Pl. XIII, Fig. 6). The rhizoids, as far as can be judged, were simple, and some at least terminated in a slightly expanded, rounded tip.

> Locality and Horizon, the same as for *H. Kidstoni*, see above, p. 567. Middle Coal-Measures.

Hepaticites Willsi, sp. nov.[1]

Dr. Wills describes the thalloid growth from Upper Coal-Measures in Staffordshire as follows:

'A thalloid growth of uncertain affinity covers certain layers in the Old Hill Marls. A typical piece is figured. . . . At first sight it appears to be formed of cutin, but since it dissolves in Schulze's macerating solution it must be of a different chemical composition. The fragments found measure as much as 10 mm. in length, and average 1·5 mm. in breadth. They branch dichotomously and repeatedly. This fact, together with the lack of differentiation of the tissue and the presence of spore-tetrads embedded in the thallus, points to the possibility of its being a primitive type of Bryophyte. Should further study support this view, these specimens will be of great interest as being the first Bryophytes recorded from the Palaeozoic rocks.'

Dr. Wills's type-specimen is refigured (Pl. XIII, Fig. 8). It is one of a large number of fragments of this species found by her. As regards the chemical composition of these fossils, the reaction with Schulze's reagent is, I think, significant, and suggests that the surface layer was uncutinized. Dr. Wills does not figure any of the spores embedded in the thallus and I have not been able to confirm her observation. I have in several instances seen spores attached to the surface, but none that were within the tissue, and because of this I am inclined to await confirmation of the presence of spores belonging to the plant. In any case, the presence of embedded spore-tetrads is not necessarily a Bryophytic character, as it is also characteristic of some of the Algae.

Each well-preserved terminal portion of a branch has a distinct notch (Pl. XIII, Figs. 9, 10, *aa*), indicating that the growing-point was carried forward in a sunken position, and that the division planes of the apical cell (or cells) were normal to the plane of the thallus: in other words, the thallus was not

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[1] Wills, L.: Geol. Mag., N.S., Dec. vi, vol. i, p. 388.

cylindrical with its growing-point sunk in a pit, but was a flat ribbon-shaped structure, with its apical cell retained at the base of a notch caused by the initially greater rate of growth of the marginal cells formed at the growing point. A dichotomy was almost certainly initiated by the division into two of the apical cell. This is strikingly suggested by the structure of some of the apices (Pl. XIII, Fig. 10). The branching appears to have been in one plane.

The thallus, constructed of approximately isodiametric cells, shows no indication of vascularization. The thallus must have been three or four cells thick, judging by the network of cell boundaries (Pl. XIII, Fig. 11). The average breadth of fifteen well-preserved fragments is 0·9 mm.; the maximum is 1·4 mm., while the minimum is 0·6 mm. There are various structures on the thallus of a rather indefinite nature: examples of one type of these are figured (Pl. XIII, Fig. 9, *s*); they suggest that small depressions with a raised border may have been present at these points.

No reproductive structures are known. It is, however, common enough to find large masses of living Hepatics growing in an entirely vegetative condition. The living species, *Riccia fluitans*, which has much the same life-form as this fossil, when growing as a submerged aquatic produces no fruits; and it is only when drier conditions prevail that such are formed.[1] The occurrence, therefore, of such abundant fragments in the shale (p. 568), suggests that the fossil may have been originally growing under similar conditions. The character of this plant and its close resemblance to *Hepaticites Langi*, to be described below, warrant its inclusion in this form-genus, even though we have not in this instance the presence of rhizoids which are found in connexion with the latter plant.

> Localities: 'Old Hill, Staffordshire', also from Granville Pit, Old Hill Station, Staffordshire.
>
> Horizon: For the latter locality, Old Hill Marls in upper 300 feet of the Etruria Marl Group, Staffordian Series, Upper Coal-Measures.

Hepaticites Langi, sp. nov.

The specimens which are included in this species were contained in the same shales from which *H. Kidstoni*, *H. lobatus*, and the fragments of *Lepidodendron* were obtained.

H. Langi consists of small dichotomously branched shoots (Pl. XIII, Figs. 12 and 13), constructed of thin-walled parenchyma, and showing, apart from a slight elongation of the cells near the axis of the shoot, no differentiation of the tissues of the thallus to form a conducting system. The shoot was constructed, in all probability, by the divisions of a single apical cell

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[1] Campbell, D. H. (1905): Mosses and Ferns, p. 24.

sunk by the initially greater rate of growth of the marginal cells in an apical notch (Pl. XIII, Fig. 14) as in *H. Willsi*, to which in general form it is very similar. It is a smaller plant; the average breadth of eleven fragments being 0·6 mm. (max. 0·8, min. 0·5 mm.). The thallus is of less robust build, and appears to be fewer cells in thickness: it is also less freely branched. Such differences might be due merely to difference in environment, but we obviously cannot assume that such was the case with any degree of confidence.

There is no doubt about the Bryophytic affinities of this plant, for, in addition to having the general appearance of an Hepatic such as *Riccia* spp. and *Aneura* spp., portions of the thallus have been found with rhizoids attached (Pl. XIII, Fig. 15). The rhizoids are not found on most of the specimens, and must have been restricted to comparatively small areas. In one specimen a group of rhizoids is situated just below a dichotomy of the thallus (Pl. XIII, Fig. 13, *rh*). In another specimen which I consider to belong to the same species, there is a small branch occupying a lateral position with respect to the main part of the thallus at the base of the branch, and on the adjoining parts of the thallus is situated a bunch of rhizoids (Pl. XIII, Fig. 16, *rh*). The somewhat unusual position of this branch in relation to the main axis may be due to some injury sustained by the plant in an earlier stage of its development. The rhizoids are simple and have smooth walls. They originated from cells which are of a darker colour than the rest of the cells in the thallus (Pl. XIII, Fig. 15, *rhc*). In some of the examples of well-preserved apices (Pl. XIII, Fig. 14), near the position of the apical cell and for a short distance behind it, there are some small, isolated, dark-coloured structures (Pl. XIII, Fig. 14). They are of the same order of size as the cells of the thallus, and in their position and relation to the growing-point suggest that they may represent mucilage producing papillae similar to those found in some of the living Hepaticae.

> Locality: Shale from Preesgweene Colliery, Preesgweene, 2¼ miles north of Oswestry, Shropshire.
>
> Horizon: 'Middle Coal Measures', Yorkian Series, Upper Carboniferous.

Any detailed comparison between the fossils which have been described above and the modern Liverworts is deferred to a later time when more specimens are described. In the meanwhile it is sufficient to call attention to the fact that there is a marked thalloid or 'frondose' character in the four species described. In *H. Kidstoni* the breadth of the axial region is large in relation to the size of its appendages; it compares, therefore, in this respect as well as in others, with such living genera as *Fossombronia* and *Blasia*: and indeed, as Professor Lang has suggested, resembles the Anacrogenae more closely than the Acrogenae. *Hepaticites Willsi* and

H. Langi in habit and in the structure of their thallus can be paralleled in the modern genera *Aneura* and *Riccia.* In regard to thallus differentiation, *H. lobatus* occupies an intermediate position between *H. Kidstoni* and the two thalloid forms. Judging by vegetative characters, one is therefore inclined to regard them all as possibly more nearly related to the Anacrogenous Jungermannieae than to the other divisions of the Hepaticae. There is no clear suggestion of any of the Acrogenae present in this series of forms. In the absence of any reproductive structures one cannot, however, come to any definite conclusions about the position they occupy in relation to the living Hepaticae.

An examination of the fossils which have been described removes all doubt as to the presence of Liverworts of a comparatively high degree of specialization in the Palaeozoic, and effectively negatives any suggestion that the Liverworts are a ' recently ' evolved group.

SUMMARY.

1. The present state of our knowledge of the fossil history of the Bryophyta, in particular of the Liverworts, is briefly reviewed.

2. A new form-genus, *Thallites,* is suggested for thalloid fossil plants of indeterminate affinity.

3. The form-genus *Hepaticites* is defined.

4. The following Upper Carboniferous fossils are described :—

(*a*) *Hepaticites Kidstoni*, sp. nov., a Liverwort of Middle Coal-Measure Age.

(*b*) *Hepaticites lobatus*, sp. nov., a species from the same horizon.

(*c*) „ *Willsi.* sp. nov., originally described by Dr. L. Wills as a thalloid organism of probable Bryophytic affinities from the Etruria Marl Group, Upper Coal-Measures.

(*d*) *Hepaticites Langi*, sp. nov. A thalloid Bryophyte, similar in general structure to *H. Willsi*, from rocks of Middle Coal-Measure Age.

5. It is suggested that these fossils may be more closely compared with the Anacrogenous Jungermannieae than with any other group of the Hepaticae.

6. These fossils demonstrate the existence of Hepaticae in the Palaeozoic.

EXPLANATION OF PLATE XIII.

Illustrating Mr. J. Walton's paper on Carboniferous Bryophyta.

Hepaticites Kidstoni, sp. nov.

1. Type specimen of leafy shoot. *o.*, cell with black contents ; *f.*, group of cells with granular contents ; *l., l.*, small leaves ; *x, x*, places where the large leaves overlap the axis, or one another. × 40.

2. Another fragment of type specimen Fig. 1 ; lettering as before.   × 40.

3. Apex of one of the large leaves seen in Fig. 1 ; lettering as before.   × 125.

4. Part of type specimen at greater magnification; lettering as before.   × 70.

Hepaticites lobatus, sp. nov.

5. Part of a vegetative shoot.   The dark bodies are probably of fungal origin.   × 40.

6. Part of another specimen. *rh.*, rhizoids.   × 40.

7. Part of another specimen at greater magnification.   *m.*, one of several adherent microspores (affinities unknown).   × 125.

Hepaticites Willsi, sp. nov.

8. Type specimen of Dr. Wills's *thalloid growth*.   × 20.

9. Fragment of another specimen.   *s.*, structures of unknown significance on thallus.   *a*, apical notch of branch.   × 24.

10. Shoot about to form a dichotomy.   × 40.

11. Cellular structure of thallus.   × 135.

Hepaticites Langi, sp. nov.

12. Small branch showing a dichotomy.   × 40.

13. Another specimen with a group of rhizoids (*rh.*).   × 40.

14. Apex of a shoot at greater magnification.   The small dark spots just below the apica notch are the supposed remains of mucilage papillae.   The branched filament on the right is probably a fungal hypha.   × 100.

15. Fragment of a shoot with a bunch of rhizoids.   *rhc.*, cells of origin of the rhizoids. A fold-over of a part of the thallus has obscured some of the details.   × 70.

16. Portion of a shoot with a small branch with a group of rhizoids at its base.   × 40.

Studies on the Tissues concerned in the Transfer of Solutes in Plants.

The Effect on the Upward Transfer of Solutes of cutting the Xylem as compared with that of cutting the Phloem.

BY

OTIS F. CURTIS.

FROM a rather extensive series of experiments, some of the results of which have already been reported (2, 3), I have obtained very definite evidence that the ringing of woody stems by the removal of the phloem tissues interferes with the upward transfer of solutes. This interference has been evident for all types of solutes for which tests were made, i. e. carbohydrates, nitrogen, and ash. Evidence for such an interference in upward transfer of solutes has been obtained by determining the effect of ringing on growth, on osmotic concentration of the tissue sap, as well as on the actual concentrations of carbohydrates, nitrogen, and ash. These ringing experiments have uniformly shown this interference in solute movement independent of the kind of plant, and independent of the season.

It seems that the simplest and most obvious explanation of these results is that the greatest amount of upward transfer occurs through the phloem tissues, and not through the xylem as has been commonly assumed. The only other explanation of the data that seems at all likely is that the ringing in some way may have altered the xylem so that it could no longer carry solutes.

That ringing does sometimes actually result in a change of the xylem, causing a partial or even complete plugging of the water-conducting vessels, is without question. There is evidence, however, that such plugging rarely occurred in the experiments reported in this series of papers, and there is also evidence that, even if such plugging had regularly occurred, this would not offer a sufficient explanation for the failure of solutes to move in approximately the normal amounts.

For the most part those who have reported plugging of vessels as the

result of ringing have not taken the precaution to protect the exposed xylem. That such failure in supplying protection very frequently results in plugging of the xylem was clearly evident when I started the first series of experiments, especially when small twigs were ringed. For this reason all ring wounds, in all experiments previously reported in this series, have been carefully protected by melted paraffin. By an oversight this precaution was not mentioned in the first paper of the series (2). The melted paraffin was usually applied at a temperature of about 90° C., the temperature depending somewhat on the temperature of the air and the size of the twig. That the heat would cause little if any injury to the surface of the xylem was evident from the fact that, unless special precautions were taken to scrape the cambium, this would very frequently begin growth under the paraffin, healing the tissue over. Similar stems without the protecting paraffin dried out and failed to heal. If the paraffin was too cool to adhere well it would become loosened from the wound, which would then dry out.

Even assuming that plugging occurred regularly, this does not prove that the check in solute transfer is due to the plugging. In every type of ringing experiment performed, consisting of about nine rather distinct conditions of treatment, it was found that if a narrow band of phloem were left intact, a band containing not over one-tenth to one-fourth the normal amount of phloem tissue, the transfer of solutes was nearly normal. It has also been repeatedly shown that but a small part of the xylem cylinder will carry sufficient water to keep the leaves apparently normal. But a small part of the phloem is sufficient to allow for approximately normal solute transfer while its complete severance causes a marked reduction, and since a small part of the xylem is sufficient to allow for approximately normal water conduction while its complete severance practically stops water movement, and as the xylem could be only partly plugged at most in these ringing experiments for there is very ready water movement; therefore any such partial plugging cannot fully account for the marked reduction in solute transfer. The more probable explanation seems to be that while water moves chiefly in the xylem, solute movement occurs chiefly in the phloem.

Though in the experiments that I have reported plugging could at most have been only partial, and though such partial plugging cannot fully explain the marked check in solute movement, there is still the possibility that the xylem may, in some other way, have been altered by the ringing so as to interfere with solute transfer. There is also the possibility that both xylem and phloem must work together. It would seem very desirable, therefore, to cut the xylem and leave the phloem intact to see whether it alone is capable of carrying solutes.

The carrying out of such an experiment involves greater difficulties

than are attendant upon ringing experiments. It is ordinarily very difficult to cut out the xylem without at the same time causing considerable injury to the phloem. Cutting the xylem not only removes a conducting tissue, but also removes the supporting tissue. A still greater difficulty to be met is that of supplying water to the tissues above the point where the xylem is cut, because the phloem is practically useless for carrying water.

In order to escape this last difficulty, the bases of well-rooted cuttings of *Ligustrum ovalifolium,* Hassk., were split longitudinally for a distance of about 10 centimetres. A piece of xylem about 1–2 centimetres long was cut out from one of the halves, the exposed inner surface of the bark coated with warm paraffin, and the gap bridged with a splinter of wood which was fastened to the surface of the split wood with hot paraffin. Though this did not make a very strong support, it did serve to keep the parts of the twig in position. All the exposed surfaces of the xylem were then coated with paraffin. Each rooted half, still attached at the upper end to a common stem, was then placed in a separate container, and the two vessels were fastened firmly together and filled with sand. A solution of sodium nitrate was poured on the sand which contained the roots of the half with the xylem cut, and water only was added to the other side. It was assumed that the water for the top would come through the xylem from the container supplied with water only, while the nitrogen, if it passed to the top, would have to move through the phloem, because a piece of the xylem had been cut out of this half. Similar split twigs were treated in a like fashion, except that the phloem instead of the xylem was cut from the half with roots watered with nitrate, while other split twigs with neither xylem nor phloem cut were used as controls. Each pair of containers had at least one plant of each type of treatment.

The plants used in this experiment had been grown in sand with low nitrogen supply, so that the leaves were distinctly yellowish green. After a week or more, those leaves of the controls which were on the side of the twig connected with the nitrate container showed a distinctly darker green colour than the others. Some of those with the phloem cut showed slight indications of darker green, while only four or five of those with the xylem cut showed this greening. These few, however, appeared more as the control stems. Examination a few days after the beginning of the experiment showed that the phloem was drying out in most of the stems where the xylem was cut, and further examination after a month or more showed that the phloem of all the stems with the xylem cut, except the few which showed the darker green leaves, had dried or rotted. The bark, stem, and roots below were perfectly healthy in these few, however. The stems and roots of those twigs with the phloem cut were all dead below the ring at this time.

One of the twigs with xylem cut which lived, together with its corresponding control twig, and the one with phloem cut were analysed for nitrogen, and though the one with xylem cut and the control showed high nitrogen, while that with the phloem cut showed low nitrogen, the data are not clearly satisfactory. Too few stems with their xylem cut lived to supply conclusive evidence, and furthermore, since the roots of the twigs with phloem cut died, due to starvation, they were thus prevented from absorbing nitrogen.

Since the phloem of these stems dried out so easily, a similar set of experiments was performed with *Bryophyllum calycinum*, Salisb., for the bark region containing the phloem of this plant is succulent and resistant to drying. After a week or ten days, the control plants showed very striking greening of the leaves on the side to which nitrate was added, while those on the opposite side of the stem, the side with roots not supplied with nitrogen, remained yellow green, very much as at the beginning. Those leaves, the petioles of which were attached half-way between the two sides, showed fairly dark green in that half of the leaf more directly above the roots in the nitrated soil, while the other half remained more yellowish green. These results were strikingly in accord with those reported by Auchter (1). The plants with the phloem cut showed little or no increased greening, while those with the xylem cut appeared very much as the controls, but the greening was delayed about a week.

These results seemed to indicate that the nitrogen could move up through the phloem when the xylem was removed, but could not move up through the xylem when the phloem was cut. Examination of the bark tissues where the xylem had been cut showed, however, that new xylem strands had developed in this region. It seemed possible, therefore, that the nitrogen had moved in this new xylem tissue. The delay in greening of the leaves in these plants seemed to add weight to such an explanation. It is also possible, however, that with deficient water the phloem could not carry the nitrogen rapidly, but that it recovered this ability when the newly developed xylem strands supplied it with water. It is evident that this type of experiment must be modified before dependable data can be obtained. It will be necessary to keep the phloem moist, and to stop the experiment before new xylem can have developed.

It might be remarked, in this connexion, that the formation of new xylem from bark tissues has also been observed in twigs of *Ligustrum* when kept from drying out long enough. In these cases, the cambium evidently does not cut off xylem tissues on the concave side and phloem on the convex, but considerable proliferation occurs and new xylem strands seem to arise in the mass of tissue.

As no more material was available for continuing this type of experi-

ment, further experiments were carried out with shoots of bushes growing outdoors. Experiments with defoliated growing shoots similar to those described in a previous paper (2) were carried out with an additional treatment in which one of the twigs of each set had the xylem severed and the phloem left intact. In order to supply water to the upper part of the shoot, the cut end of the xylem was placed in a small phial of water fastened to the main stem. In every case, however, the phloem withered. Evidently it is very poorly adapted for carrying water, not supplying enough even to keep itself alive.

The experiment was therefore modified so as to keep the phloem constantly moist. A split cork with a single hole for the stem was fitted around the stem just below the region where the xylem was to be cut and secured with melted paraffin. The bark was split on opposite sides of the stem for a distance of 2–5 centimetres above the cork, loosened from the xylem, and separated so that the stem could be bent over and the xylem cut off. The end of the cut xylem was then passed out through the slit in the bark, and 1–2 centimetres cut off. The twig was then straightened, a thin-walled tube, 8–12 centimetres long, and open at both ends, passed down over the stem, and fitted securely to the cork. This tube was then filled with water which completely covered the wound, keeping the exposed bark wet and supplying water to the xylem for the shoot above. Just before slipping the tube over the stem its base was dipped into hot paraffin, so that it would make a watertight seal with cork.

A similar defoliated twig, with the phloem instead of the xylem cut, was in like manner encased in a tube of water, and a third twig was defoliated and left as a control. The control, however, was not encased in a tube of water. None of these wounds was covered with paraffin because the water in the tube was expected to protect the exposed surfaces. The length of the shoot was taken and measurements again made, usually after one-day intervals. Data obtained from such an experiment with *Philadelphus pubescens*, Loisel., are presented in Table I. In this, and in all subsequent experiments reported in this paper, the stems were attached to the growing plant.

The data clearly show that cutting the phloem has interfered with continued growth, though the xylem is left intact, while cutting the xylem and leaving the phloem has allowed for almost normal growth. There seemed to be a remote possibility, however, that solutes may have been given off the cut end of the xylem below into the water, from which they may have been absorbed by the cut xylem above. In order to exclude this as a possibility other experiments were performed with *Philadelphus*, as well as with *Berberis Thunbergii*, D.C., and *Rhus typhina*, L., in which the tubes were thoroughly rinsed with distilled water once a day. In some of the experiments the additional precaution was taken to seal, with hot

TABLE I. *Effect of cutting xylem as compared with that of cutting phloem on growth of defoliated shoots of* Philadelphus.

Cut part of each treated shoot enclosed in tube of water.   Growth expressed in millimetres. Experiment started June 13, 1924.

Set No.	1	2	3	4	5	6	Averages.	Rel. to control as 100.
Gain in length in first day, June 13–14.								
Control	3	6	2	—	3	3	3·4	100
Phloem cut	2	5	6	2	3	3	3·5	103
Xylem cut	5	9	2	5	5	3	4·8	141
Gain in length second and third days, June 14–16.								
Control	22	28	24	—	25	13	22·4	100
Phloem cut	3	1	2	4	1	3	2·3	10
Xylem cut	14	16	dry	13	20	14	15·4	69
Gain in length fourth and fifth days, June 16–18.								
Control	15	28	29	—	41	12	25·0	100
Phloem cut	1	1	1	2	3	3	1·8	7
Xylem cut	24	17	—	16	28	21	21·2	85
Gain in length sixth day, June 18–19.								
Control	10	15	20	—	17	2	12·8	100
Phloem cut	0	0	0	0	0	1	0·2	1
Xylem cut	4	15	—	12	1	4	7·2	56
Total gain in length in six days, June 13–19.								
Control	50	77	75	—	86	30	63·6	100
Phloem cut	6	7	9	8	7	10	7·8	12
Xylem cut	47	57	—	46	54	42	49·2	77

paraffin, the cut end of the xylem from which solutes might exude.   Such precautions, however, made no measurable differences in the responses. Data from an experiment with *Rhus*, in which all of the tubes were rinsed daily, and half of the cut ends were sealed, are presented in Table II.

It would seem from these experiments that materials necessary for growth cannot move through the xylem when the phloem is absent, but can move through the phloem when the xylem is absent.   In the experiment with *Philadelphus* there seemed to be no significant differences between the different treatments at the end of the first day, but with *Rhus*, and in all other experiments where measurements were made the day after beginning the experiment, the differences seemed to be significant, though not so striking as on the second day and those following.   A summary of these and other experiments is presented in Table III.

These data add still further evidence that cutting the phloem interferes very distinctly with continued growth, even though the xylem is left intact, while cutting the xylem and leaving the phloem has not caused very great reduction in growth.   When cutting the xylem, the phloem in many cases

TABLE II. *Effect of cutting xylem as compared with that of cutting phloem on the growth of defoliated shoots of* Rhus.

Cut part of each treated shoot enclosed in a tube of water. Growth expressed in millimetres. Experiment started June 26, 1924.

Set No.	1	2	3	4	5	6	Aver-age.	Rel. to control as 100.
			Gain in length first day, June 26–27.					
Control	11	5	5	8	6	5	6·7	100·0
Phloem cut	2	1	4	4	2	0	2·2	32·8
Xylem cut	9	2	5	2	2	2	3·7	55·7
			Gain in length second day, June 27–28.					
Control	7	7	8	13	8	13	9·3	100·0
Phloem cut	3	3	3	1	2	2	2·3	24·7
Xylem cut	10	7	5	7	6	8	7·2	77·4
			Gain in length third day, June 28–29.					
Control	18	18	19	17	17	19	18·0	100·0
Phloem cut	5	7	6	5	3	2	4·7	26·1
Xylem cut	26	18	24	12	19	16	19·2	106·7
			Gain in length fourth and fifth days, June 29–July 1.					
Control	broken	22	29	34	33	29	29·4	100·0
Phloem cut	broken	5	9	8	6	6	6·8	23·1
Xylem cut	25	19	26	26	8	13	19·5	66·2
			Total gain in five days, June 26–July 1.					
Control	—	52	61	72	64	66	63·0	100·0
Phloem cut	—	16	22	18	13	10	15·8	25·1
Xylem cut	70	46	60	47	35	39	49·5	78·6

TABLE III. *Effect of cutting the xylem as compared with that of cutting the phloem on the growth of defoliated shoots of various plants.*

Cut part of each treated shoot enclosed in a tube of water. Growth expressed in millimetres. Data given are averages in most cases of five to seven or more stems.

	Philadelphus Series 1		Philadelphus Series 2		Philadelphus Series 3		Berberis		Rhus Series 1		Rhus Series 2	
		Rela-tive.		Rela-tive.		Rela-tive.		Rela-tive.		Rela-tive.		Rela-tive.
				Gain in length in millimetres during first two days.								
Control	25·8*	100·0	18·6	100·0	15·4	100·0	25·8	100·0	16·0	100·0	12·8	100·0
Phloem cut	5·8*	22·5	8·8	47·3	4·4	29·3	8·4	32·5	4·5	28·1	4·9	38·3
Xylem cut	20·2*	78·3	20·0	107·5	11·6	75·4	15·0	58·1	10·9	68·1	9·4	73·5

Total gain in length in millimetres to end of experiment.

Duration.	6 days.		7 days.		6 days.		5 days.		5 days.		12 days.	
Control	63·6	100·0	105·3	100·0	70·3	100·0	73·4	100·0	63·0	100·0	318·2	100·0
Phloem cut	7·8	12·3	19·7	18·7	10·5	14·9	11·0	15·0	15·8	25·1	54·5	17·1
Xylem cut	49·2	77·3	47·4	45·0	41·3	58·8	38·5	52·5	49·5	78·5	229·8	72·2

* Gain in length for first three days, as no measurements were taken after two days.

was partly injured.   Often from one-half to three-fourths of the phloem was broken off while endeavouring to cut out the xylem, yet growth was in all cases much better than in those stems in which the phloem was cut, even though this latter operation was very simple, and resulted in no injury to the xylem.   It seems pretty clear, therefore, that some substances necessary for growth cannot move through the xylem, but can move through the phloem.

A few preliminary determinations of the influence of these treatments on the water content and the total soluble sugar[1] content were made. Such data obtained from the shoots of *Philadelphus* are presented in Table IV.   The leaves which had opened during the experiment were removed from the control stems and from those with the xylem cut, in order that the tissues might be more comparable to those of the ringed stems which had developed no new leaves.   Since the shoots of the controls and those with the xylem cut had grown larger than those that were ringed, their bases were cut off, leaving the upper part of practically the same length as the ringed stems.

TABLE IV.   *Effects of cutting xylem as compared with those of cutting phloem on the growth and the water and sugar content of defoliated shoots of* Philadelphus.

	No. of Stems.	Average total gain in length per stem in 6 days, June 13–19. mm.	Total green wt. grm.	Total dry wt. grm.	Percentage dry wt.	Total sol. sugar. mg.	Sugar % green wt.	Sugar % dry wt.
Control	5	63·6	12·676	1·3712	10·8	15·4	0·12	1·12
Phloem cut	6	7·8	12·576 *	1·1280 *	9·0	< 0·4 *	< 0·03	< 0·35
Xylem cut	5	49·2	12·192	1·1312	10·8	26·6	0·22	2·03

* Corrected for five stems.

From this table it is evident that the ringing had not interfered with water absorption, for the water content of the ringed stems is higher than in either of the other treatments.   This was also evident from the turgor of the shoots.   They were very brittle and easily broken, while both the controls and the stems with the xylem cut were distinctly tougher and not so easily broken.   It is also evident that the ringing had hindered the upward movement of sugar, for its content was very low.   The failure to receive sugar was, in this case, probably the chief reason for failure to continue growth.

Determinations were also made of the water and sugar contents of terminal buds from the first experiment with *Rhus*.   Whatever new leaves

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[1] I am indebted to Mr. Daniel Clark for making these sugar determinations.

had developed during the experiment were included with the bud in each case. The data are presented in Table V.

TABLE V. *Effects of cutting the xylem as compared with those of cutting the phloem on the growth and the water and sugar content of defoliated shoots of* Rhus.

Set No.	2	3	4	5	6	Averages.	Rel. to control as 100.
Total gain in mm. in five days, June 26–July 1, 1924.							
Control	52	61	72	64	66	63·0	100
Phloem cut	16	22	18	13	10	15·8	25
Xylem cut	46	60	47	35	39	49·5	72
Total green wt. of each bud at end of experiment, gm.							
Control	1·164	1·530	2·038	1·337	1·724	1·559	100
Phloem cut	0·728	0·590	0·700	0·637	0·405	0·552	35
Xylem cut	1·003	1·831	1·614	0·866	1·020	1·294	83
Dry wts. of buds as percentages of fresh wts.							
Control	22·0	21·3	21·5	22·7	24·1	22·3	100
Phloem cut	16·4	16·1	16·8	18·3	18·5	17·2	77
Xylem cut	18·8	17·8	20·4	20·3	23·2	20·1	90
Total soluble sugar in each bud, mg.							
Control	3·8	5·2	5·4	6·2	4·4	5·0	100
Phloem cut	3·7	1·8	3·3	6·9	2·6	3·7	74
Xylem cut	4·6	5·2	5·5	4·9	3·3	4·7	94
Sugar percentage of green wt. in each bud.							
Control	0·33	0·34	0·27	0·46	0·26	0·33	100
Phloem cut	0·87	0·31	0·47	1·05	0·64	0·67	203
Xylem cut	0·46	0·28	0·34	0·57	0·32	0·39	118
Sugar percentage of dry wt. in each bud.							
Control	1·49	1·60	1·23	2·04	1·05	1·48	100
Phloem cut	5·34	1·90	2·81	5·93	3·46	3·89	263
Xylem cut	2·45	1·59	1·67	2·79	1·39	1·97	133

The effects on growth and water content are similar to those reported for *Philadelphus*, that is, cutting the phloem has very distinctly reduced the growth and increased the water content, but as contrasted with the data in the previous table, the percentage of sugar over that in the buds and leaves of the control and xylem-cut stems, expressed either in relation to fresh weight or dry weight, has been very much increased by ringing. The fact that the percentage of sugar in the ringed shoots was more than twice that in the controls, while the dry weight was nearly 25 per cent. less, is an interesting one. It would seem, in this case, that the failure of the ringed stems to grow was due, not to a lack of carbohydrate, but to the deficiency of some other material which cannot move through the xylem.

It is probable that the difference between the sugar contents in the

Philadelphus series and the *Rhus* series are not due entirely to the fact that they were different kinds of plants.   In fact, an incomplete set of analyses of a different series of *Philadelphus* indicated a condition more nearly comparable to that in *Rhus*.   A more probable explanation can be based on the conditions existing at the time of beginning the experiment. In the first *Philadelphus* series, the bushes were growing on the north side and close to the wall of a building.   The branches experimented with were on the side next to the wall, and were partly shaded by overhanging eaves.   Furthermore, the experiment was started during cloudy and rainy weather.   Under such conditions it would be expected that the carbo-hydrate content of the shoots would be low to begin with, and that the sugar supply would therefore tend to be a limiting factor for growth.   For the incomplete set of *Philadelphus*, on the other hand, the branches were on the exposed side of the bushes, and the experiment was started in brighter weather ; and in the experiment with *Rhus* the plants were growing in the open, and the experiment was begun on a day when there was no rain, and there was much more sunshine.

It is interesting to note that in both sets of analyses the sugar content of the shoots with the xylem cut is considerably in excess of that in the controls.   It seems probable that the accumulation is, at least, partly due to the fact that the growth had been somewhat checked.   This check may have been in part due to decreased water absorption, because the ability of cut xylem to absorb water seems to fall off considerably, especially after a day or two.

More complete analyses under varying conditions would probably show different materials, acting as limiting factors, depending on the conditions of the experiment and the kind of plant used.

In order to determine whether or not the ringing had resulted in plugging of the xylem, stems from each of the series here reported were tested.   Though microscopical examination showed no signs of plugging, it is very possible that plugging might not be easily observed in cross or longitudinal sections.   For this reason, the freedom of movement of water, and of coloured solutions through the ringed parts of the stems, was tested by cutting out this part of the stem, including a piece of stem 1–5 centimetres long, both above and below the ring.   No differences in the rate of flow of water, or of solutions of various dyes through these ringed stems, were observable when compared with similar pieces of stem taken from the control twigs.   Such tests were made both when the solutions were allowed to move by gravity alone, and when placed under suction. The dye often passed through the ringed or control stems under suction in less than a minute.   On the other hand, pieces of those stems from which a section of xylem was removed showed no movement of dye past the region where the xylem was cut, even when placed under suction for

periods running up to fifteen hours. This is a pretty good indication that new xylem vessels had not developed as had occurred in the former experiments. Furthermore, the fact that the growth in the shoots with the xylem cut practically equalled that of the controls from the very first day is additional evidence that translocation had not taken place through newly formed xylem. On the other hand, when the phloem was cut, the growth of shoots was appreciably retarded within the first twenty-four hours in all the experiments except one, and in this one the retardation was very apparent in the second period.

DISCUSSION.

A very large number of ringing experiments of various types, and performed under various conditions, have uniformly indicated that the complete interruption of the phloem very appreciably hinders the upward transfer of solutes. The two most obvious interpretations are, that the phloem is the path through which the greatest amount of solute movement occurs, or that the ringing in some way alters the xylem so that it can no longer carry solutes. A number have not been willing to accept the first interpretation on the ground that ringing experiments cannot be relied upon in studies on upward translocation because of the possible injury to the xylem. Dixon (4) has very logically carried his criticism beyond those experiments which are directed to determine what tissues carry solutes upward, and includes also those experiments in which an attempt is made to determine what tissues carry foods downward. He is perfectly justified in the conclusion that if ringing experiments cannot be relied upon for determining the tissues concerned in upward transfer, neither can they be relied upon for determinations of downward transfer. It might be added, however, that his most direct evidence in favour of downward movement through the xylem is based on experiments, where not only is one of the tissues cut and a foreign substance introduced, but that particular tissue itself which it is hoped to demonstrate as the conducting tissue is the one that is cut. In the experiments on ringing and on cutting the xylem here reported, first one and then the other tissue is left intact. It is true that Dixon offers as evidence for the downward transfer of solutes through uninjured stems the downward transfer of a stimulus in sensitive plants. But at least two unproved assumptions are made, that some solute of the nature of a hormone carries the stimulus, and that this is carried only in the xylem.

The question raised by Dixon as to whether the phloem is capable of carrying the amount of material as fast as it evidently must be carried is a pertinent one, and one which at present cannot be easily answered. The suggestion that the xylem is better fitted than the phloem to carry solutes—

to the potato tuber for example—does not, however, simplify matters. In the first place, calculations of the cross-sectional area of the conducting tissues of potato rhizomes, based on drawings kindly furnished by Dr. Artschwager, indicate that the xylem has an even smaller cross-sectional area than has the phloem, and in the second place, to my knowledge, sugars have never been demonstrated to be present in the vessels of potato xylem.

Some seem to think that the only possible method of solute movement is by means of mass flow of solution through open tubes, and since solutions cannot be forced through phloem tissues, while they can very easily be forced through the xylem, the latter is assumed to be the chief conducting tissue. A great deal of evidence is available, however, which indicates that the phloem is efficient in carrying foods downward, and yet this tissue certainly does not allow for ready mass flow of solutions, therefore any such explanation would seem to have to be abandoned. Diffusion alone most certainly cannot account for the movement. The suggestion of de Vries (5), that streaming and rotation of the protoplasm and solution within the living cells are responsible, seems to be the most likely explanation. Such streaming would very much hasten the movement of solutes from one end of a cell to the other in both directions, leaving for diffusion only the movement across occasional membranes. The claim has been made that such streaming is the result of injury, and never normally occurs, but it has been fully demonstrated that it is perfectly normal in *Elodea, Nitella*, and such aquatic forms, and it may normally occur in the phloem tissues of most plants. It would be desirable to have more evidence as to its occurrence, the rate of movement, and the factors affecting it. I do not propose to discuss this phase of the question in detail in the present paper, but the small amount of evidence which is available indicates that translocation is dependent on living cells, and tends to favour the protoplasmic streaming hypothesis. If such protoplasmic streaming within the cells does account for the downward transfer of solutes through the phloem, it could easily account for the upward transfer also, because the total amount of solute material that moves down is as a rule very much in excess of that moving up.

<div align="center">SUMMARY.</div>

Though many types of ringing experiments previously reported have uniformly shown that ringing interferes with the upward transfer of solutes, it had not been definitely shown that this hindrance may not have been due to plugging or other alteration of the xylem resulting from the ringing.

Evidence is discussed which indicates that plugging could have been but partial at most, and that partial plugging could not fully account for the hindrance in solute movement.

Experiments in which the effects of cutting the xylem, as compared with those of cutting the phloem, on solute transfer are described.

By the use of divided stems, where water is supplied to the tops by one set of roots, and nitrogen by the other, a few data were obtained indicating that if the roots supplied with nitrogen are connected with the top by the xylem only, there was little transfer of the nitrogen to the tops, while if they were connected by a strip of phloem only, considerable transfer occurred. The data were incomplete and not very satisfactory, however, for when the xylem was cut, the phloem strip usually dried out, or else new xylem strands developed in the time allowed for the experiment.

Experiments making use of defoliated growing shoots, however, supplied data showing that when the shoots were attached to the parent plant by the xylem only they failed to grow, evidently because some material necessary for growth could not move through the xylem, while those connected by a strip of phloem made a growth nearly as good as did the stems with both xylem and phloem, indicating that the phloem could carry these materials.

Determinations in three distinct series showed that the water content of the ringed shoots exceeded that of the controls and those with the xylem cut, indicating that the failure to grow was not due to lack of water.

Sugar determinations showed in one series that the ringed shoots had an excessively low sugar content, as compared with the controls and the stems with their xylem cut, indicating that the growth had been limited by lack of sugar, while in another series the ringed shoots, though they had less total sugar, had, on the percentage basis, a sugar content considerably in excess of those of the other treatments, indicating that some other factor was limiting in this series.

It seems that solute movement, both upward and downward, occurs chiefly through the phloem tissues, and that this is hastened by streaming movements within the living cells.

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Some Salient Lines of Specialization in Tracheary Pitting.

I. Gymnospermae.

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With Plates XIV and XV and two Figures in the Text.

INTRODUCTION.

THE study of the comparative anatomy of the Pteridophyta and Gymnospermae suggests that the specialization of tracheary pitting tends to progress along several rather clearly defined lines. The most significant of these appear to be: (1) a general tendency towards the replacement of scalariform structures by nearly isodiametric pits, and (2) a tendency towards a reduction in the number of pits in the facets of secondary tracheides.

In the metaxylem of the Filicales, the scalariform reticulate thickenings [1] frequently acquire overhanging margins, and thus form transversely elongated bordered pits, which tend at times to divide into smaller and more nearly isodiametric bordered pits. A tendency towards the replacement of primitive scalariform by multiseriate pitting is observable in the peripheral secondary xylem of certain Sigillarias, and appears to be intensified in the wood of a number of the Sphenophyllales and Calamariales. The specialization of the tracheary pitting seems to move backward from

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[1] De Bary recognized two main categories of tracheary elements : (1) tracheae with fibrous thickenings, and (2) pitted tracheae. The fibrous thickenings may be spiral, annular, or reticulate. Reticulate thickenings, the meshes of which are elongated transversely and are arranged on one surface of the wall in a row one above another, are designated as scalariform reticulate. It should be emphasized in this connexion, however, that it is only the relative size of the unequally thickened parts of the membrane which gives rise to a general distinction between reticulately thickened and pitted membranes. Therefore, it is difficult in many cases to determine whether specific tracheary elements should be classified as scalariform reticulate or as scalariform pitted.

the later towards the earlier formed portion of the xylem. In other words, the first-formed tracheary elements tend to retain relatively primitive types of secondary thickenings, and there is a sequence of increasing structural specialization in the successively formed tracheides. In the more highly differentiated forms, the zone of transitional structures is confined to tracheides in relatively close proximity to the protoxylem.

In the secondary xylem of the Pteridospermae or Cycadofilices and Cordaitales, the tracheary pitting usually is of the alternating and more or less compressed circular type. Scalariform bordered pitting is restricted, except in the Cladoxyleae, Protopityeae, and such Cordaitean forms as *Dadoxylon protopityoides*, to tracheides in relatively close proximity to the protoxylem. That the multiseriate pitting of the Cycadofilices and Cordaitales may primitively have originated from a scalariform type of sculpture is inferred from analogy with the derivation of similar tracheary pitting in the Lepidophytineae, Sphenophyllales, and Calamariales, and from the sequence of structural changes in the first-formed tracheides of the stele.

In discussing the sequence of structural changes in the inner portion of the cauline vascular cylinder of *Cordaites*, most palaeobotanists refer to sections of well-preserved stems of *Dadoxylon (Cordaites) Brandlingii*. Through the courtesy of the Redpath Museum of McGill University, the writer secured the privilege of studying Dawson's and Penhallow's slides of this plant. As noted by Scott,[1] Seward,[2] Penhallow,[3] and others, the large discoid pith of *D. Brandlingii* is jacketed by a broad zone of spiral and transitional tracheides. In passing centrifugally from the protoxylem, through the transitional region, into the secondary xylem, one may observe the following sequences of structural changes in the successively formed tracheary cells (Text-fig. 1). The loosely coiled fibrous thickenings of the inner protoxylem are succeeded by more compactly wound spirals, and these, in turn, by scalariform reticulate thickenings. In subsequently formed elements, the transversely elongated meshes of the scalariform facets first acquire conspicuous borders and later break up into smaller oblong, oval, or more or less circular bordered pits. The form and the arrangement of the bordered pits vary considerably within the limits even of a single cell or facet; the scalariform being replaced by both opposite and alternate bi- or tri-seriate pitting. Although the alternate seriation tends to become dominant in the later-formed elements of the secondary wood, 'vestiges' of scalariform and opposite pitting are of not infrequent occurrence in tracheides formed at some distance from the transitional region.

This sequence of structural changes suggests an 'ontogenetic' epitome

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[1] Scott, D. H.: Studies in Fossil Botany, 2nd ed. London, 1909.
[2] Seward, A. C.: Fossil Plants, vol. iii. Cambridge, 1917.
[3] Penhallow, D. P.: A Manual of the North American Gymnosperms. Boston, 1907.

or recapitulation of successive evolutionary modifications in the derivation of multiseriate from scalariform pitting, and is so interpreted by Penhallow [1] and Seward.[2] Such transitional sequences, of a more or less condensed type, are stated to occur in other Cordaitean forms and in various representatives of the Pteridospermae or Cycadofilices.

It is of interest, in this connexion, to study the tracheary pitting of the higher Vasculares and to determine how complete and reliable a record of
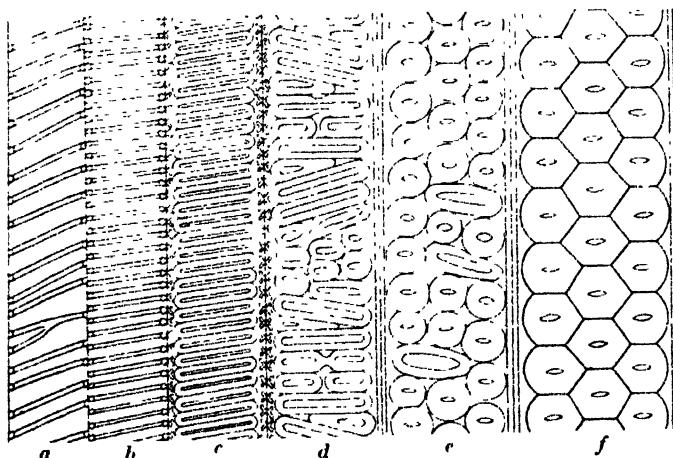
TEXT-FIG. 1. *Dadoxylon (Cordaites) Brandlingii.* Radial longitudinal section of the inner portion of the caulme xylem, showing the sequence of transitional structures in a considerably abbreviated form. *a*, tracheide with loosely coiled fibrous thickenings; *b*, tracheide with compactly wound spiral thickenings; *c*, tracheide with scalariform bordered pits; *d* and *e*, tracheides with transitional bordered pitting; *f*, later-formed secondary tracheide, showing seriation of bordered pits in the more profusely pitted portion of a radial facet.

successive evolutionary modifications is retained in their inner zone of transitional tracheides. With this object in view, the writer has made an extensive survey of the tracheary pitting in the primary and secondary xylem of the Bennettitales, Cycadales, Ginkgoales, Coniferales, Gnetales, and Angiospermae. The Gymnosperms will be discussed in the following pages.

MATERIAL AND METHODS.

Statements in the literature concerning the tracheary pitting of the Bennettitales were verified by an examination of well-preserved specimens of Bennettites and Cycadeoidea. In addition, material was secured from 187 species of fifty-three extant genera of the Gymnosperms:

[1] Penhallow, D. P.: loc. cit., pp. 38–40.
[2] Seward, A. C.: loc. cit., p. 251.

	Genera.	Species.
Cycadaceae	9	15
Ginkgoaceae	1	1
Taxaceae	9	23
Araucarieae	2	8
Abieteae	9	86
Taxodieae	9	10
Cupresseae	11	39
Gnetaceae	3	5

In studying the sculpture of the transitional tracheides of extant Gymnosperms, it was found advisable to soak short sections of stems in solvents of oleo-resins before embedding in nitro-cellulose, and to treat thin radial and tangential serial sections in a 1 per cent. solution of hot (65° C.) caustic soda before staining in Heidenhain's haematoxylin and mounting in glycerine jelly. Such preparations were supplemented by macerations stained in haematoxylin or nigrosin.

BENNETTITALES AND CYCADALES.

As Wieland[1] has shown, scalariform pitting tends to predominate in the cauline centrifugal xylem of many Mesozoic Cycadeoideae. In such forms as *Cycadeoidea Dartoni, C. Paynei, C. Wielandi, Bennettites Gibsonianus,* &c., the inner zone of spiral and scalariform reticulate tracheides is succeeded by tracheary elements with scalariform bordered pits in their radial facets. These transversely elongated bordered pits not infrequently tend, on the one hand, to break up into smaller bordered pits of opposite and alternate seriation and, on the other hand, to contract into a single row of oblong, oval, or nearly circular bordered pits. Thus, the tracheary pitting of these plants resembles that which occurs in the secondary xylem of Cladoxyleae, Protopityeae, and *Dadoxylon protopityoides.*

In the oldest available stems of *Stangeria paradoxa* and *Zamia floridana,* the tracheary derivatives of the cambium form a broad zone of elements with scalariform reticulate thickenings (Pl. XIV, Fig. 1). That these elements may be succeeded, in the peripheral secondary wood of older and thicker stems, by tracheides with scalariform and transitional types of bordered pits is suggested by the occurrence of such pits in the outermost secondary tracheides of fairly stout stems of *Microcycas Calocoma,* and in the centripetal metaxylem of petioles of *Stangeria, Zamia,* and *Microcycas* (Pl. XIV, Figs. 4 and 5). Although the bordered pits in the latter tissue are predominantly scalariform, they tend at times to be replaced by smaller pits of opposite and alternate seriation.

The tendency towards the replacement of scalariform by circular bordered pits in the later-formed tracheides of the cauline centrifugal xylem

[1] Wieland, G. R.: American Fossil Cycads, vol. ii. Carnegie Inst. of Washington, 1916.

appears to be intensified at least in certain representatives of the Cycadales, e. g. *Dioon, Cycas, Macrozamia, Encephalartos*, and *Ceratozamia*. For example, in the large columnar stems of *Dioon spinulosum*, the bulk of the secondary wood is composed of tracheides with more or less numerous bordered pits of the type illustrated in Pl. XIV, Fig. 6. Scalariform and intermediate forms of sculpture are confined to the relatively wide zone of transitional elements upon the inner margin of the vascular cylinder. The following sequences of structural changes occur in the successively formed tracheary elements of this transitional region. The scalariform reticulate tracheides acquire more conspicuously bordered meshes (Pl. XIV, Fig. 2), which subsequently break up into smaller bordered pits of opposite and alternate seriation (Pl. XIV, Fig. 3). These more or less circular bordered pits increase in size and are reduced in number in the later-formed tracheides. Their seriation in the more profusely pitted portions of the facets is alternate (Pl. XIV, Fig. 6).

Similar, but considerably more compact, transitional sequences occur in the centripetal xylem of the petioles of *Dioon, Cycas, Macrozamia, Encephalartos*, and *Ceratozamia*. Tracheides with spiral and scalariform reticulate thickenings are succeeded by elements with scalariform and transitional bordered pitting (Pl. XIV, Fig. 7), and these, in turn, by tracheides with alternating. nearly circular bordered pits (Pl. XIV, Fig. 8). In other words, the tracheary pitting in the large later-formed elements of the petiolar centripetal metaxylem tends to be of the alternating and nearly circular type, whereas that which occurs in homologous tracheides of the petioles of *Stangeria, Zamia*, and *Microcycas* is predominantly scalariform or transitioi ̣ ̣.

In the Bennettitales and Cycadales, as in the Sphenophyllales and Calamariales, there appears to be a strong tendency towards the replacement of scalariform by more nearly isodiametric pits. There is an additional tendency towards a reduction in the number of bordered pits in the radial facets of the peripheral secondary tracheides. Furthermore, the specialization of the tracheary pitting seems to move backward from the later towards the earlier-formed xylem, but the first-formed tracheides tend to retain relatively primitive types of secondary thickenings, even in the more highly differentiated forms. The specialization of the cauline centrifugal xylem tends to be paralleled by that of the petiolar centripetal metaxylem.

GINKGOALES AND CONIFERALES.

The tendency towards a reduction in the number of bordered pits in the facets of the secondary tracheides, which is observable in certain of the Cycadofilices, Cordaitales, and Cycadales, is intensified in the Ginkgoales and Coniferales. The circular bordered pits in the secondary xylem of

extant representatives of these orders rarely are contiguous or flattened by close crowding except in the Araucarieae. In *Ginkgo*, the Taxaceae, Abieteae, Taxodieae, and Cupresseae, the seriation in the more profusely pitted surfaces fundamentally is ' opposite ', whereas in the Araucarieae it characteristically is ' alternate '.

As indicated in Text-fig. 2, the sequence of structural changes in the transitional tracheides of the Coniferales is entirely unlike that which occurs in homologous elements of the Cordaitales (Text-fig. 1) and Cycadales.
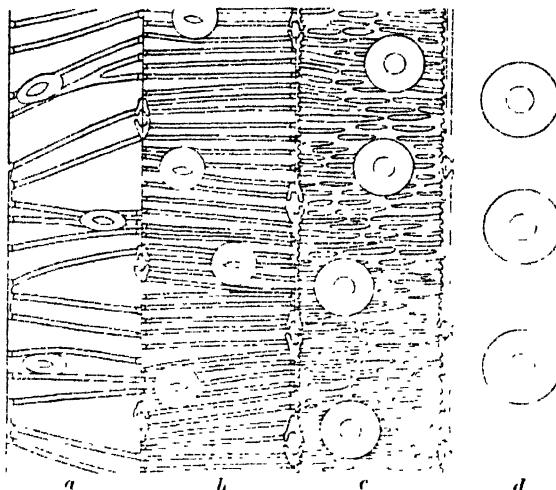
TEXT-FIG. 2. *Abietean Conifer.* Radial longitudinal section of the inner portion of the cauline xylem, showing typical sequence of structural changes. *a*, primary tracheide w... !....., coiled fibrous thickenings and small oval bordered pits; *b*, primary tracheide with compactly wound spiral thickenings and widely separated circular bordered pits; *c*, tracheide of ...er-formed metaxylem, showing large circular bordered pits and shallow slit-like groove , *d*, secondary tracheide with larger circular bordered pits.

More or less circular and isolated bordered pits tend to be present even in the spirally thickened tracheary elements of the protoxylem (Pl. XIV, Figs. 9–18; Pl. XV, Figs. 18–26). These pits increase in diameter in succeeding tracheides (Pl. XV, Figs. 24 and 21). In the metaxylem, the tenuous and closely coiled fibrous thickenings tend to coalesce and to form a reticulum in portions of the facets which are not occupied by the bordered pits (Pl. XV, Fig. 24). However, the narrow, slit-like, unthickened areas of this reticulum do not differentiate into scalariform and transitional types of bordered pitting, but become occluded, giving rise first to shallower grooves and ultimately to uniformly thickened portions of the secondary membrane ; compare Text-figs. 1 and 2.

Such facts as these suggest that in the relatively narrow and obviously much reduced transitional zones of the higher Gymnosperms, the circular type of bordered pitting has tended to work back into the protoxylem, and

has resulted in the elimination of typical scalariform and transitional types of bordered pitting. More or less transversely elongated bordered pits are of sporadic occurrence in the inner transitional elements of *Agathis*, *Araucaria*, and *Ginkgo*. These pits tend at times to divide into smaller pits of opposite and alternate seriation, as well as to contract into single circular bordered pits, and thus simulate vestiges of the scalariform and intermediate types of bordered pitting which occur in the transitional zones of the Cordaitales, Cycadales, and lower Vasculares. Their absence in other genera is suggestive of a higher degree of specialization of the transitional tracheides. However, it should be noted, in this connexion, that there is a considerable element of uncertainty in attempting to distinguish cenogenetic from truly palingenetic structures in the highly specialized transitional tracheides of the Ginkgoales and Coniferales.

The bordered pits which are formed on both the radial and the tangential facets of the spirally and reticulately thickened tracheides may be separated more or less widely (Pl. XIV, Figs. 11, 13 ; Pl. XV, Figs. 19, 25, 27), or aggregated in an opposite or alternate seriation (Pl. XIV, Figs. 9 and 17). Although they commonly are less numerous in the earlier than in the later formed tracheides of the primary wood, their frequency of distribution in the latter elements tends in general to simulate that of the enlarged circular bordered pits in the secondary tracheides (Pl. XV, Figs. 24 and 25). In other words, where the pits in the secondary wood are abundantly developed, e. g. certain Araucarieae, the tracheary elements of the metaxylem tend to be profusely pitted, and, on the contrary, where the pits in the secondary tracheides are less numerous, e. g. many Abieteae, Taxaceae, Taxodieae, and Cupresseae, those in the metaxylem tend to be widely separated. In the more profusely pitted forms, the seriation of the pits in the transitional tracheides is more or less heterogeneous, both opposite and alternate arrangements occurring even within the limits of a single cell or facet (Pl. XIV, Figs. 9 and 17). This is true regardless of whether the seriation in the peripheral secondary wood is opposite or alternate.

GNETALES.

The tracheary pitting of *Ephedra* resembles that of the Coniferales. The circular bordered pits in the secondary tracheides tend to be more or less widely separated, but may at times be aggregated in an opposite or alternate biseriate grouping. As shown in Pl. XV, Fig. 23, circular bordered pits occur in the spirally thickened elements of the primary xylem. Furthermore, the spaces between the more closely approximated fibrous thickenings become occluded without giving rise to typical scalariform and transitional types of bordered pitting.

The more salient lines of structural modification in the tracheary elements of the Gnetales are those that are associated with the differentia-

tion and specialization of vessels. In *Ephedra*, the vessels are composed of tracheide-like segments which are provided with several circular perforations in their terminal portions. As indicated by the investigations of Thompson [1] and others, these circular perforations appear to have originated from bordered pits by a reduction of the bordering areas and the elimination of the septa and tori. More or less complete transitional stages between typical bordered pits and perforations tend to be epitomized in the first-formed secondary xylem. Vessels of the *Ephedra* type are of not infrequent occurrence in the innermost xylem of *Gnetum*. In passing centrifugally from this zone, the vessels rapidly increase in size, and, as they do so, the numerous circular perforations coalesce to form large oval or elliptical openings. At least in the structurally less specialized species of *Ephedra*, the vessels appear to be confined to the secondary xylem, whereas in the more highly differentiated forms of *Gnetum* they are present in the primary metaxylem (Pl. XV, Fig. 22). As will be shown in a subsequent paper, the differentiation and specialization of vessels in the Dicotyledons tend to progress centripetally from the secondary xylem into the metaxylem and ultimately into the protoxylem.

It is evident, accordingly, that in the Gnetales, as in the Ginkgoales and Coniferales, the primary xylem is structurally of a highly modified type, and an accurate record of successive structural changes in the evolution of the circular type of bordered pit is not retained in the inner zone of transitional tracheides. The differentiation and specialization of vessels, however, tend to be more or less clearly epitomized in the first-formed portions of the steles of *Ephedra* and *Gnetum*.

DISCUSSION.

In the Cordaitales, Bennettitales, and Cycadales, as in the Lepidophytineae, Sphenophyllales, Calamariales, and Cycadofilices, the first-formed tracheides of the stele appear to retain relatively primitive types of secondary thickenings, and successive structural changes in the derivation of nearly isodiametric bordered pits from scalariform reticulate thickenings tend to be more or less fully and accurately epitomized in succeeding tracheides. On the contrary, in extant representatives of the Ginkgoales, Taxaceae, Araucarieae, Abieteae, Taxodieae, Cupresseae, and Gnetales, the circular bordered pits have worked back into the earlier-formed portions of the primary xylem and typical scalariform and transitional types of bordered pitting are eliminated.

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[1] Thompson, W. P. : The Anatomy and Relationships of the Gnetales. I. The Genus *Ephedra*. Ann. Bot , xxvi. 1077-1104, 1912. Independent Evolution of Vessels in Gnetales and Angiosperms. Bot. Gaz., lxv. 83-90, 1918.

The highly ·modified structure of the transitional tracheides renders difficult the task of securing reliable recapitulationary evidence concerning the origin of the types of bordered pitting which occur in the peripheral secondary tracheides of the higher Gymnosperms. Thus, the occurrence of opposite—in association with alternate—pitting in transitional zones of *Agathis* and *Araucaria* does not afford a cogent argument for asserting that the Araucarian seriation is a modification of the Abietean, and conversely the presence of alternate—in association with opposite—pitting in homologous zones of *Ginkgo* and *Pinus* does not prove the Abietean seriation necessarily to be derived directly from the Araucarian, or even from a Cordaitean alternating multiseriate arrangement. Nor does the occurrence of heterogeneous seriations in the secondary xylem of various Mesozoic plants of putative coniferous affinities afford a reliable basis for assuming that opposite seriations necessarily are derived from alternate or vice versa. As suggested by Seward,[1] such seriations may represent less stereotyped arrangements, and may be derived directly from scalariform pitting. In the secondary xylem of the Cladoxyleae, Protopityeae, *Dadoxylon protopityoides*, and various Bennettitales, the scalariform or transversely elongated bordered pits tend to break up into smaller pits of both opposite and alternate seriations, as well as to contract into a single row of more or less compressed oblong. oval, or circular bordered pits. Furthermore, in the transitional zones of many of the lower Vasculares, scalariform pitting tends to be succeeded by multiseriate pitting of heterogeneous seriation (Text-fig. 1 and Pl. XIV, Fig. 3), the alternate arrangement becoming dominant in the later-formed tissue. That dominantly opposite, as well as prevailingly alternate, seriations may be derived independently from scalariform pitting or from heterogeneous multiseriate arrangements is, therefore, by no means improbable. It should be emphasized, in this connexion, that similar types of bordered pitting appear to have originated in different groups and at different times through convergences or parallel development. This is indicated, for example, by the occurrence of scalariform and derived types of pitting in such remotely related groups as the Filicales, Lepidophytineae, Sphenophyllales-Calamariales, and Bennettitales-Cycadales.

The highly specialized structure of the primary xylem in extant representatives of the Ginkgoales, Coniferales, and Gnetales raises the question whether similar specializations have occurred in other groups of the vascular plants, and renders essential a more comprehensive and detailed study of the transitional tracheides of the Vasculares in general, and of the Cordaitales, the Angiospermae, and various Mesozoic plants of putative coniferous affinities in particular.

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[1] Seward, A. C. : Fossil Plants, vol. iv, p. 184. Cambridge, 1919.

CONCLUSIONS.

A study of the comparative anatomy of the Pteridophyta and Gymno-spermae reveals several putative lines of specialization in tracheary pitting and suggests that:

1. The differentiation of tracheary pitting tends in general to move backward from the later towards the earlier formed portion of xylem.

2. In the lower Gymnosperms and Pteridophyta, the first-formed tracheary elements retain relatively primitive types of secondary thicken-ings, and successive structural changes in the derivation of nearly isodia-metric bordered pits from scalariform reticulate thickenings tend to be more or less fully and accurately epitomized in succeeding tracheides.

3. In extant representatives of the Ginkgoales, Coniferales, and Gnetales, the circular bordered pits have worked back into the earlier formed portions of the primary xylem, and typical scalariform and transi-tional types of bordered pitting are eliminated from the transitional zone of the stele.

4. However, in the Gnetales, successive stages in the differentiation and specialization of vessels are more or less accurately epitomized in the first-formed portion of the stele.

5. Similarities in tracheary pitting may be due to convergences or parallel development—as indicated, for example, by the occurrence of scalariform and similar derived types of pitting in such remotely related groups as the Filicales, Sphenophyllales-Calamariales, and Bennettitales-Cycadales—and, therefore, may not be indicative of close genetic relation-ships.

6. In the Coniferales, owing to the highly modified structure of the transitional tracheides, there is no reliable recapitulationary evidence to indicate whether Abietean pitting is derived from Araucarian or vice versa, or whether both seriations originated independently from less stereotyped arrangements or from scalariform pitting.

7. The tracheary pitting of the transitional tracheides, particularly in the Cycadofilices, Cordaitales, and various Mesozoic plants of putative coniferous affinities, deserves more critical and detailed investigation than it has received heretofore.

8. The highly specialized structure of the primary xylem in the Ginkgoales, Coniferales, and Gnetales may prove to be of considerable significance in discussions concerning the phylogeny and relationships of the higher Gymnosperms and Angiosperms.

ACKNOWLEDGEMENTS.

EXPLANATION OF PLATES XIV AND XV.

Illustrating Professor I. W. Bailey's paper on Gymnospermae.

PLATE XIV.

Fig. 1. *Zamia floridana.* Radial longitudinal section of cauline centrifugal xylem, showing scalariform reticulate tracheides. × 125.

Fig. 2. *Dioon spinulosum.* Radial facet of cauline transitional tracheide, showing scalariform bordered pits. × 600.

Fig. 3. *Dioon spinulosum.* Radial facet of later-formed cauline transitional tracheide, showing the replacement of scalariform by multiseriate bordered pitting. × 600.

Fig. 4. *Zamia integrifolia.* Longitudinal section of petiolar centripetal xylem, showing sequence of structural changes. × 340.

Fig. 5. *Microcycas Calocoma.* Facet of tracheide from petiolar centripetal metaxylem, showing scalariform and alternate biseriate bordered pitting. × 600.

Fig. 6. *Dioon spinulosum.* Radial facet of tracheide from later-formed secondary xylem, showing size, form, and arrangement of the bordered pits. × 600.

Fig. 7. *Cycas revoluta.* Longitudinal section of petiolar centripetal xylem, showing sequence of structural changes. × 330.

Fig. 8. *Ceratozamia mexicana.* Longitudinal section of petiolar centripetal xylem, showing sequence of structural changes. × 230.

Fig. 9. *Saxegothaea conspicua.* Facet of primary tracheide, showing circular bordered pits and intervening fibrous thickenings. Note heterogeneous seriation of the bordered pits. × 600.

Fig. 10. *Ginkgo biloba.* Facet of primary tracheide, showing large circular bordered pits and intervening transverse fibrous thickenings. × 600.

Fig. 11. *Podocarpus Nageia.* Facet of primary tracheide, showing large circular bordered pits and loosely coiled fibrous thickenings. × 600.

Fig. 12. *The same.* Bordered pits shown in section. × 600.

Fig. 13. *Torreya californica.* Facet of spirally thickened primary tracheide, showing widely separated circular bordered pits. × 600.

Fig. 14. *The same.* Bordered pits shown in section. × 600.

Fig. 15. *Araucaria Bidwillii.* Somewhat transversely elongated bordered pits in spirally thickened primary tracheide. From axis of cone. × 600.

Fig. 16. *The same.* Bordered pits shown in section. × 600.

Fig. 17. *Agathis alba.* Facet of primary tracheide, showing circular bordered pits and intervening fibrous thickenings. The seriation of the bordered pits is both opposite and alternate. × 600.

Fig. 18. *Pseudotsuga taxifolia.* Radial longitudinal section of primary xylem, showing widely separated circular bordered pits and closely coiled fibrous thickenings. × 600.

PLATE XV.

Fig. 19. *Pinus Strobus.* Facet of spirally thickened primary tracheide, showing widely separated circular bordered pits. × 600.

Fig. 20. *The same.* Bordered pits shown in section. × 600.

Fig. 21. *Larix decidua.* Radial facet of tracheide from mature secondary wood, showing large bordered pits. × 600. Compare Fig. 24.

Fig. 22. *Gnetum species.* Radial section of the metaxylem, showing numerous circular perforations in spirally and reticulately thickened vessel. × 320.

Fig. 23. *Ephedra Gerardiana.* Circular bordered pits in the radial and tangential facets of spirally thickened primary tracheides. × 600.

Fig. 24. *Larix decidua.* Radial longitudinal section of primary xylem (left) and first-formed secondary wood right), showing smaller circular bordered pits in primary tracheide with spiral and reticulate fibrous thickenings. × 600.

Fig. 25. *Chamaecyparis thyoides.* Radial longitudinal section of primary xylem (left) and first-formed secondary xylem (right), showing circular bordered pits in spirally thickened primary tracheide. × 600.

Fig. 26. *The same.* Sectional view of bordered pits in the primary xylem. × 600.

Fig. 27. *Pseudolarix Kaempferi.* Circular bordered pits in primary tracheides with spiral and reticulate fibrous thickenings. × 600.

BAILEY—TRACHEARY PITTING.

BAILEY — TRACHEARY PITTING.
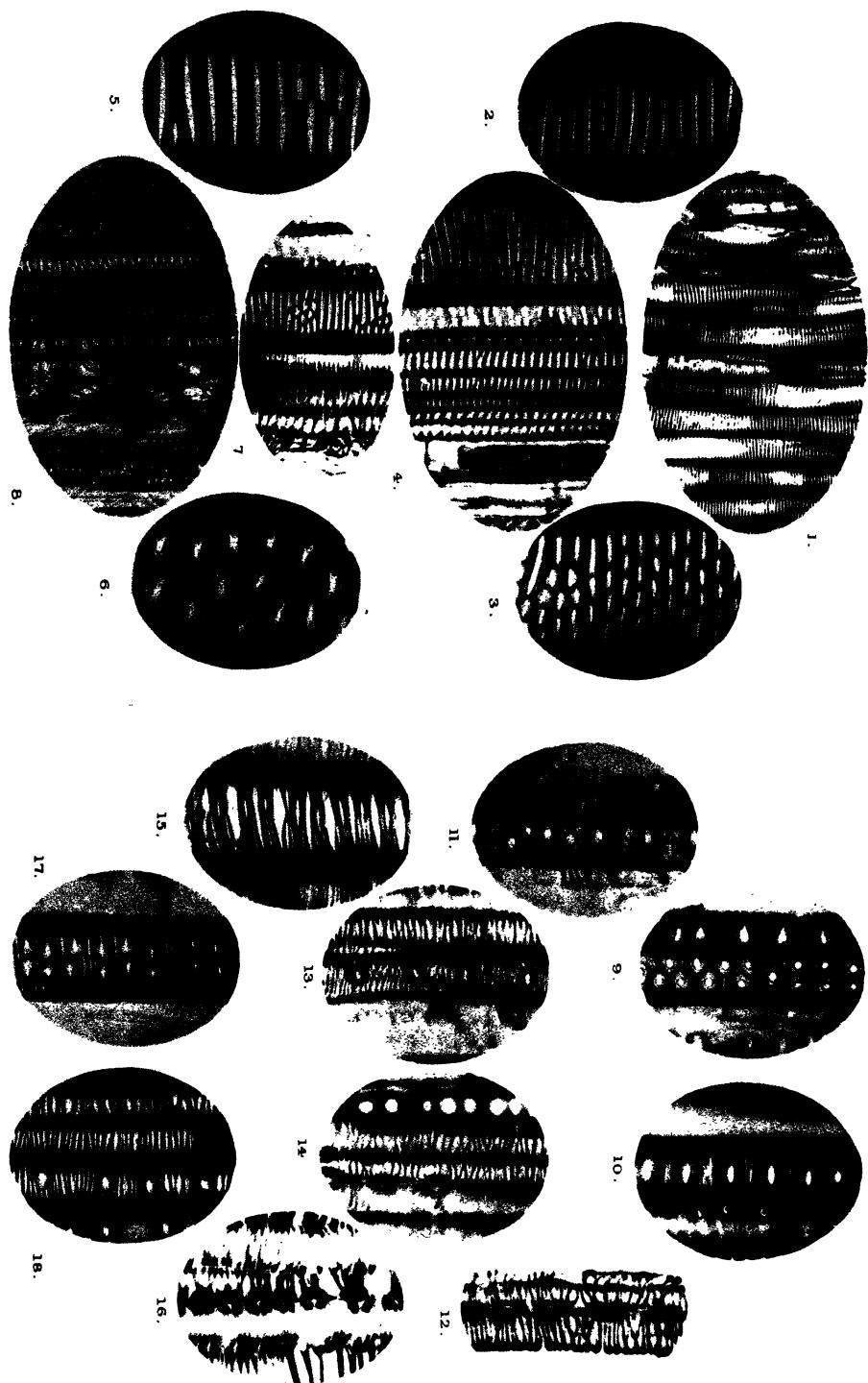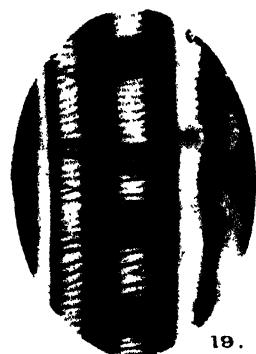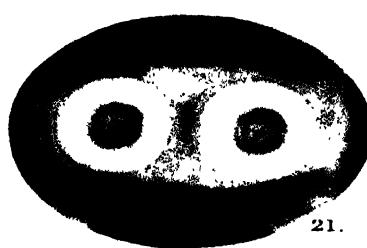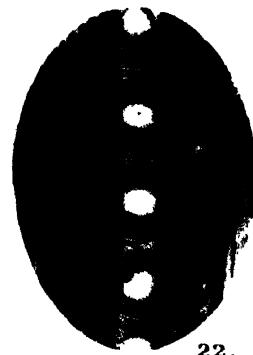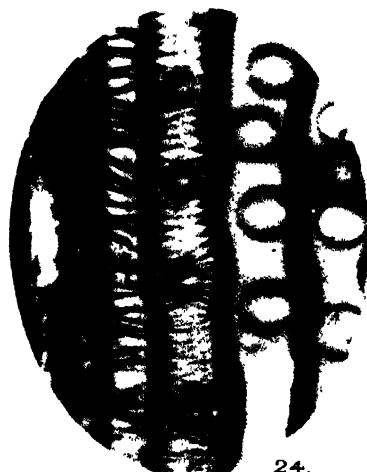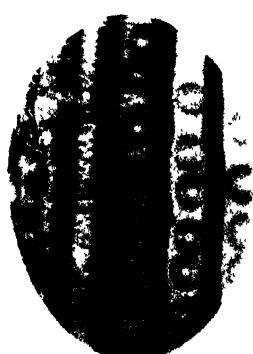
On the Leaf Structure of Juncus.

BY

R. S. ADAMSON.

With thirty Figures in the Text.

CONTENTS.

INTRODUCTION.

THE present investigation of the leaf structure of the British species of *Juncus* arose out of a general study of the genus begun some years ago. In the course of this work certain features of the leaf were noted that seemed of sufficient interest to be treated separately, and, though many of the facts have been dealt with by others, it is hoped that the additional evidence that is now brought forward will help towards confirmation of the general thesis propounded.

In the present account the details of histological structure are not considered. These have been well studied, and, for the most part, are well known. It will be sufficient here to mention the work of Douval-Jouve (7 and 8), who paid special attention to the vascular septa in the leaves of certain species, and to the accounts of the anatomy given by Buchenau (5 and 6). A rather full account of the anatomical structure of the European species of *Juncus* from a systematic standpoint has been given by Blau (4).

HISTORICAL.

Of works dealing more especially with the features under consideration at present, mention must be made of some of the more important. As long ago as 1855, Irmisch (12) made a study of the general morphology of these plants. He studied and clearly described the formation of the shoot system and the relations of the various axes to one another. Also he described the leaf succession in various species, and finally proved that the cylindrical leaf found in many is truly a leaf, and not a continuation of the stem. He further demonstrated the leaf nature of the so-called ' sterile stems '.

Buchenau (5) in 1890 studied the leaf structure in a large number of plants in the family, and concluded that the leaf structure can, in all cases, be derived from the flat dorsiventral type which is found in *Luzula* and in some species of *Juncus*. From comparative studies he derived the cylindrical types from this by a suppression, more or less complete, of the upper surface. He figures (l. c., Pl. II) several leaves in section which show different stages in this suppression. The same conclusion was reached by Goebel (9, 10, 11), who studied the leaves both comparatively and developmentally. His conclusions are brought together and summarized in 1913, when he deals with the ' unifacial ' leaf. He describes and figures a number of plants with this type of leaf, and deals especially with the arrangement of vascular bundles. In *Juncus* he shows from a study of the region of the junction of the limb and sheath that the radially symmetrical arrangement of the former is derived from a dorsiventral structure by the suppression of the adaxial surface (9, l. c., Fig. 293, p. 283). In the transition region the two surfaces are clearly distinguished by the absence of chlorophyll in the parenchyma below the adaxial surface. He further shows that the equitant leaf of *Iris* is formed in exactly the same manner as these unifacial leaves, and strongly contests the view of Massart (14), for example, that this form is due to concrescence of the two sides of the leaf. Again, he regards the unifacial cylindrical leaf as being a derived one, and not a primitive condition.

Laurent (13) has studied the embryology and germination of Juncaceae. While most of his work deals with the very early stages of development, which are not described here, he demonstrated that the first leaf produced after the cotyledon has the same form in all the species; this first leaf is bifacial.

In recent years Mrs. Arber (1, 2, and 3, &c.) has made a number of studies of the Monocotyledon leaf from a quite different point of view. She deals with the morphology of the leaf and concludes that in Monocotyledons it is a phyllode structure. This is based on anatomical evidence.

She regards the cylindrical radially symmetrical leaf found in many Liliaceae and Iridaceae as a petiolar phyllode. She quotes Buchenau's figures (5) of *Juncus* in support of her theory (1). The theory, however, does not materially affect the questions of leaf structure that are considered here, and detailed consideration of her results is unnecessary.

DESCRIPTIONS OF STRUCTURE.

Of the twenty-three species of *Juncus* that occur in Britain, eighteen have been examined more or less in detail. A description of all of these
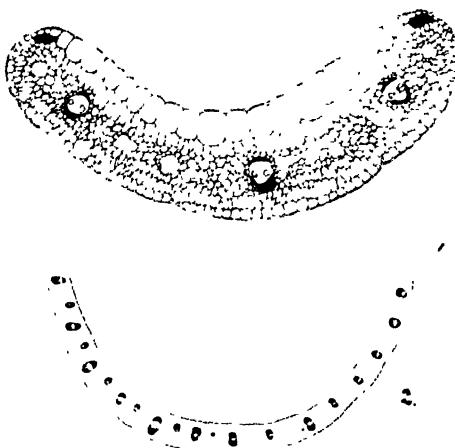
FIGS. 1 and 2. 1. Transverse section of leaf of *J. bufonius*, showing bifacial structure. × 80. 2. Transverse section of sheath of *J. effusus*. × 19. Fibres are black in both figures.

is, however, unnecessary, as it would involve much repetition and restatement of well-known facts. Instead, it is proposed to describe a few selected examples as types of the structures developed, and in these only to emphasize the features not previously fully described. The species chosen as types are *J. bufonius*, *J. effusus*, *J. maritimus*, *J. subnodulosus*, and *J. squarrosus*.

J. bufonius (Fig. 1).

Very little need be said of this plant. It has been described and figured by Buchenau (5), Pl. II, Fig. 3 and others. The leaf is bifacial and the limb forms a direct prolongation of the sheath. All through, the bundles are arranged in an arc, and all have the same orientation. There is no trace at all of reversed bundles. The upper surface is marked by an epidermis composed of large thin-walled cells, and is without stomata; it is in sharp contrast with the lower epidermis, which has many stomata, and is composed of small cells much cuticularized. Below this lower epidermis

are parenchyma cells with many chloroplasts, while those adjacent to the upper large-celled epidermis are either devoid of chlorophyll or contain very little. Towards the tip the leaf becomes smaller, but the structure remains the same ; the vascular bundles become reduced to three, and finally only one persists at the extreme point. This type of leaf occurs throughout the life of the plant. In the seedling, the first leaves produced are just like those formed later, though smaller and with only three
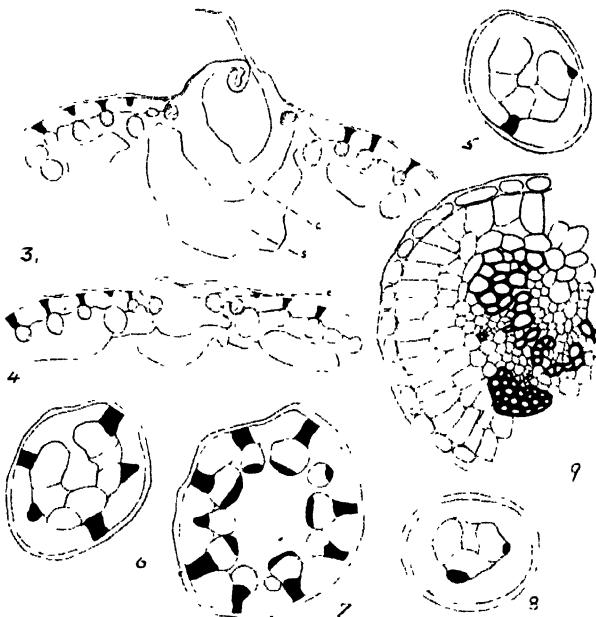
Figs. 3–9. *J effusus*. 3 Transverse section at top of sheath, showing reduced adaxial surface. *c.*, colourless parenchyma below adaxial epidermis ; *s.*, cavity between parenchyma and stellate pith. × 15. 4. As 3, but higher up. The adaxial surface and colourless parenchyma are still more reduced. × 15. 5, 6, and 7. Sections at tip from above downwards. × 55. Bundles at tip in a horseshoe open adaxially. In 7 a ring has been formed round the central pith. Fibres are black. 8. Section of the limb of cataphyll. × 80. The structure is like that at the tip (Fig. 5). 9. Small portion of 8 enlarged, showing the bundles in contact. × 215. The fibres are on the abaxial side.

vascular bundles. In the first differentiation at the apex in all the leaves three bundles are formed first. The following species, which have been examined, agree in all essential features with the above : *J. tenuis, J. trifidus* (cf. Goebel (11)), *J. compressus, J. Gerardi, J. capitatus.*

J. effusus.

This plant is so well known that it needs no general description. The only leaf which has a fully developed limb is that subtending the inflorescence. This has a structure that is perfectly symmetrical radially and

identical with that of the aerial portions of the stem. The sheath has, however, a dorsiventral structure with the bundles in an arc (Fig. 2). A study of the region of junction of the sheath and limb leads to exactly the same conclusions as those reached by Goebel, and this plant is in entire agreement with his description of *J. glaucus* (Figs. 3, 4). No details need be added here. In this region, where the adaxial surface becomes reduced and finally eliminated, there is no sudden change in position or branching of the vascular bundles. They maintain their position relative to the abaxial surface. and the ring arrangement is produced as the result of suppression of the other one. Further evidence in support of this origin of the radially symmetrical, or, to use Goebel's (11) terminology, ' unifacial ' leaf, is obtained by a study of the tip portions of the leaf. or of the small limb that is formed by the uppermost cataphylls. At the leaf-tip the number of bundles becomes very much less, and hence their arrangement can be seen more readily. A section cut quite close to the tip, though circular in outline, reveals in the arrangement of the vascular bundles a dorsiventral structure, in the form of a horseshoe open to the adaxial side (Fig. 5). Further. there are fibres connecting the abaxial bundle to the epidermis, but not in the case of the others. Fig. 6 represents the structure a short distance farther back from the tip, and shows the same dorsiventral arrangement. Farther down (Fig. 7) the number of bundles increases and the horseshoe becomes closed, and the radial symmetry is reached. At the same time the pith region increases in size. A very similar structure is seen in the small limb produced in the cataphyll (Fig. 8). Here also the bundles are in a horseshoe with the opening towards the adaxial side. In the cataphyll the pith is differentiated from the other parenchyma, while they are continuous at the tip of a normal leaf. In a cataphyll, or at the tip of a leaf, the bundles are very close together and not sharply delimited by a sheath (Fig. 9). These facts show that, even in those plants which have the cylindrical type of leaf, there is at the first initiation a dorsiventral structure which is thus revealed at the tips. There is no trace here of an adaxial epidermis ; the outline is circular, and the epidermis and chlorophyll-containing parenchyma are uniform all round.

A study of the leaves of the seedling has been made in this plant. In the young stages all the leaves have a fully developed limb at the top of the sheath. This, however, shows a progressive change of form from the first produced. The first leaves formed are bifacial and channelled above. In structure they agree in all essentials with those described above in *J. bufonius.* They have the same differentiation of the two surfaces and the same arrangement of the vascular bundles (Fig. 10). The leaf is relatively thicker. Subepidermal fibres occur at the margins at the points where the two forms of epidermis meet. This type of leaf is only found in the first two or three produced by the seedling plant. The subsequent

leaves are almost cylindrical in outline, though with a groove along the adaxial side. Fig. 2 shows a section of such a leaf. The leaf is now a tubular structure, but its bifacial structure is still obvious. Along the adaxial groove is the large-celled epidermis, below which is parenchyma without chlorophyll. This, however, here occupies only a relatively small
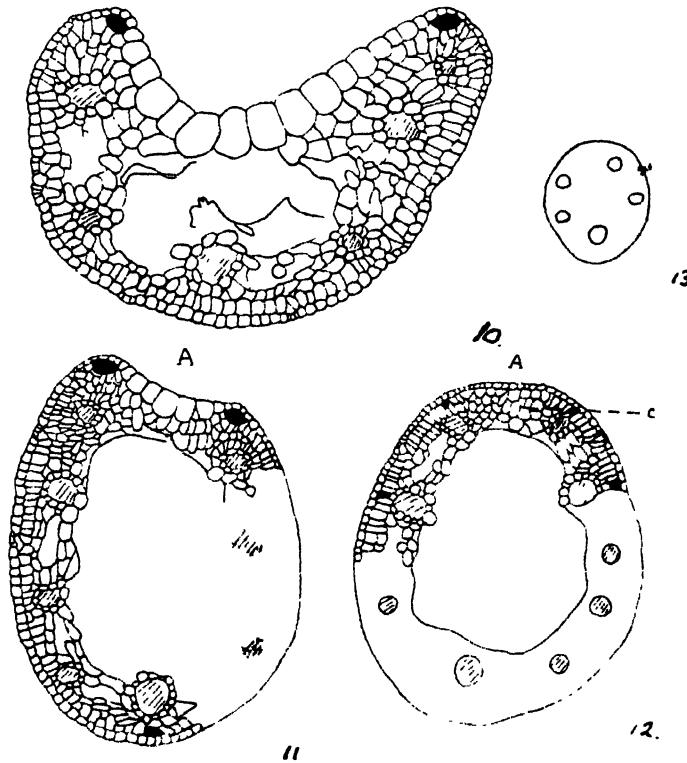
FIGS. 10–13. *J. effusus.* 10. Transverse section of first leaf of seedling showing bifacial structure. × 105. 11. Section of leaf of seedling older than 10. Bifacial leaf with adaxial surface much reduced. × 105. A is adaxial side. 12. Section of later leaf of seedling. Epidermis uniform but colourless parenchyma (*c.*) on adaxial side, A. × 105. Fibres are black in Figs. 10, 11, 12. 13. Section of leaf in apical bud of seedling. × 80. Vascular bundles arranged in a U; the largest is on the abaxial side.

fraction of the surface in section : the abaxial surface is very much extended. Subepidermal fibres are again present at the points of junction of the two surfaces. The vascular bundles instead of being in an arc are now in the form of a rather deep horseshoe.

Subsequent leaves formed have externally a quite cylindrical radial limb, but these reveal a dorsiventral structure, though this is less obvious (Fig. 12). The epidermis is uniform all round, and like that of the abaxial side of the first leaves. The bundles are almost in a ring, and each is

associated with a strand of subepidermal fibres. Fibres are not present except in relation to the bundles. On the adaxial side there is a small area of parenchyma that is without chlorophyll, and hence sharply marked off from that forming the tissue in the rest of the section.

A consideration of this series of leaves seems to leave little room for doubt that the views of Buchenau and Goebel are correct, that the unifacial structure is attained by suppression of the adaxial face. In this last-described leaf (Fig. 12) the colourless parenchyma on the adaxial side represents the subepidermal tissue of the bifacial condition. The adaxial epidermis has been suppressed, but the internal tissues are still developed.

The bifacial character of this kind of leaf is well brought out by a study of its development. Fig. 13 is a section of such a leaf in a very young stage while still enclosed in the apical bud. The procambial strands have been differentiated and are clearly in the form of a horseshoe, the opening being towards the adaxial side. The section is from the middle of a leaf limb which measured about two millimetres in length.

The type of leaf shown in Fig. 12 is the last on the seedling which has a developed limb. Subsequently the mature condition with sheathing cataphylls is reached.

It may be mentioned here that in these leaves on the seedling plant the central region is hollow. There is none of the characteristic stellate pith of the adult.

Other species agreeing in all essential features with *J. effusus* are *J. conglomeratus, J. inflexus (glaucus), J. balticus,* and *J. filiformis.* The last two have hollow leaves without pith. *J. filiformis* has a limb developed on one or more of the basal sheaths. Seedlings of *J. conglomeratus* show exactly the same stages in the leaf as those described for *J. effusus.*

J. maritimus.

The leaf structure of this plant has not been described by either Goebel or Buchenau. It has a fully developed cylindrical limb in the upper basal leaves. The limb is solid—that is, filled with tissue, not hollow or with merely a stellate pith. A section of the limb shows a structure quite different from that seen in *J. effusus.* The epidermis and chlorophyll-containing parenchyma are uniformly developed all round, but the vascular bundles are not radially arranged, instead they show a distinct dorsiventrality (Fig. 14). The main bundles are some distance from the epidermis, and are arranged in a horseshoe form with a small opening towards the adaxial side. In the centre is an elliptical or rather pear-shaped region of pith. At the abaxial side of this horseshoe is a median bundle which is placed farther from the epidermis than the others. The vascular bundles in this leaf are very numerous, and the smaller ones which

are nearer the periphery show the same arrangement. Fig. 14 is taken from the base of the limb of a young leaf; in the mature leaf the limb becomes somewhat flattened, but the flattening is not in any definite direction: it may be lateral or dorsiventral or oblique (Fig. 15). The transition region from the sheath shows exactly the same features as are seen in *J. effusus.* The sheath is much more massive, and has a much
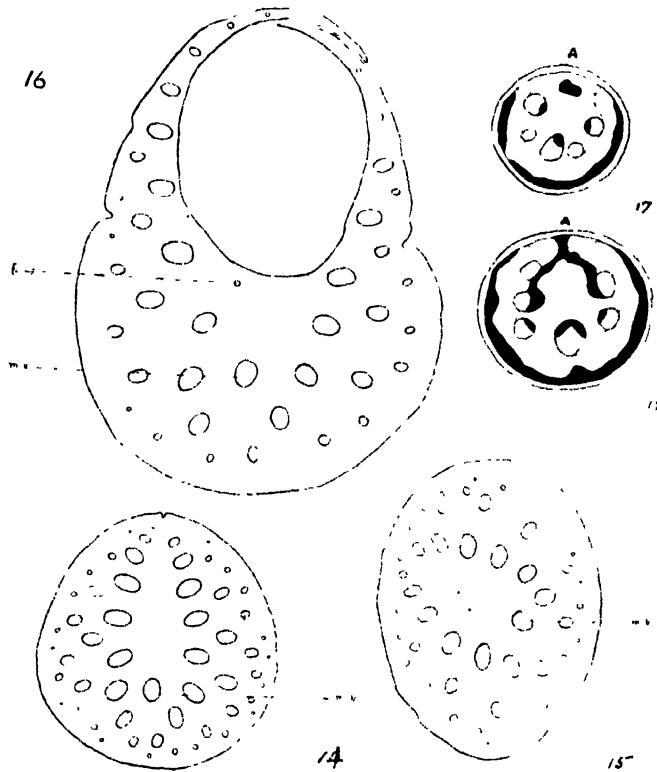
FIGS. 14–18. *J. maritimus.* 14. Section of limb just at top of sheath showing the dorsi-ventral arrangement of the bundles. × 15. *m.b.*, median bundle   15. Section of limb showing flattening in a plane oblique to the bundle arrangement. × 15. 16. Section of sheath about ⅔ up. × 15. *m.b.*, median bundle ; *f.*, fibres. 17. Section at tip of leaf showing arrangement of bundles and subepidermal fibres. × 52. 18, as 17, but farther behind tip; the horseshoe arrangement is quite clear. × 52. A, adaxial side.

thickened central region (Fig. 16 ; cf. Fig. 2). The median bundle is clearly differentiated in the sheath by its position farther from the abaxial surface. In the sheath, strands of fibres without conducting tissues occur close to the adaxial side (*f.*, Fig. 16). While the leaf as a whole shows this dorsiventral arrangement of its vascular bundles, it is not surprising to find that it persists at the tip, where an arrangement very like that seen in *J. effusus* occurs (Figs. 17, 18). The leaf-tip is very hard, due to the

presence of subepidermal fibres that are developed, especially along the abaxial side. *J. acutus* agrees in all the essential features of its structure with *J. maritimus*.

J. subnodulosus (obtusiflorus).

The limb of the leaf of this plant is cylindrical and hollow, except where crossed by the vascular septa. The bundles are arranged almost or quite radially round the circumference (Fig. 19). In a young leaf,
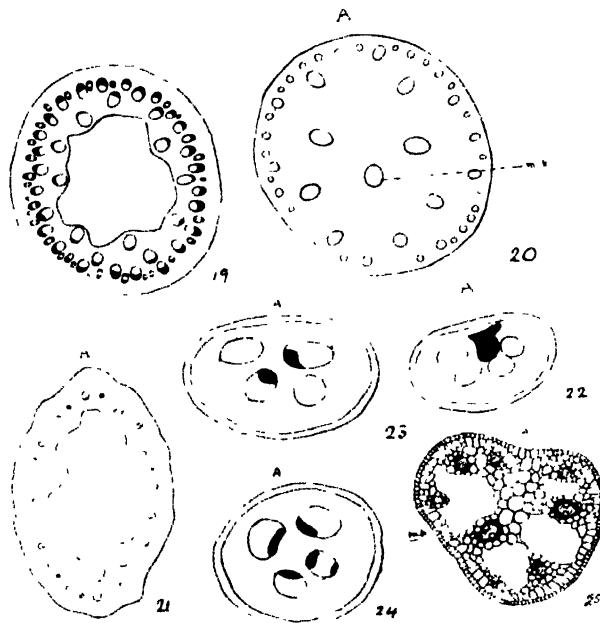
FIGS. 19-25. 19, 20. *J. subnodulosus.* 19. Section of mature leaf. Practically radially symmetrical structure. × 16. 20. Section of very young leaf. The main bundles are clearly dorsiventral. × 26. 21-4. *J. articulatus.* 21. Section of mature leaf. Radially symmetrical, but laterally compressed × 15. 22. Section at tip of leaf. Dorsal flattening, bundles in an arc. × 52 23 and 24. Successive stages farther back, showing closure of the ring of bundles. × 52. 25. *J. bulbosus.* Section of leaf. Bifacial structure with small area of adaxial epidermis. × 51. A, adaxial side; *m.l.*, median bundle.

however, before the central hollow is formed, a different structure is found which is essentially the same as that seen in *J. maritimus* as regards the larger bundles which lie nearer the centre. The small peripheral bundles are symmetrically placed (Fig. 20). This dorsiventral arrangement also occurs at the leaf-tip. In the closely allied species, *J. articulatus*, the tip structure is even more striking. Fig. 21 is a cross-section of the mature leaf of this plant, and Fig. 22 is one cut at the extreme tip. Here the leaf is dorsally flattened ; the vascular bundles are arranged side by side in an open horseshoe. A strand of fibres, that continues up to the point, lies on

the adaxial side of the bundles ; from the tip successive sections downwards show the gradual formation of the ring arrangement (Figs. 23, 24) and the appearance of the circular outline.   In this plant, even in the young leaf, there is no distinct median bundle as there is in *J. subnodulosus*.   *J. silvaticus* agrees in all essential features with these plants.   The small *J. bulbosus* (*supinus*), which is closely allied to these, has a clearly dorsiventral structure
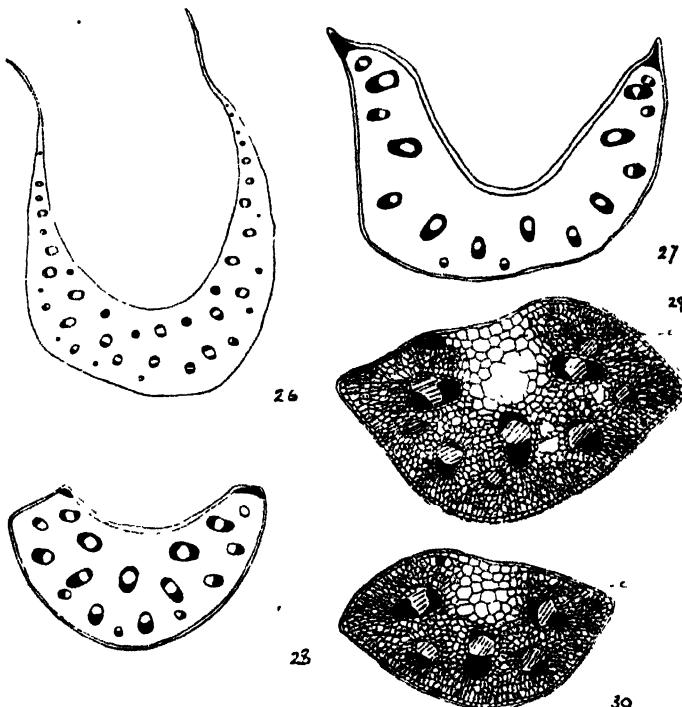
FIGS. 26–30. *J. squarrosus.* 26. Section of sheath showing arrangement of bundles and strands of fibres (black).   × 16.   27. Section of lower portion of limb.   Bifacial structure with adaxial side concave.   × 32.   28. Section at middle of limb.   Adaxial surface reduced and leaf thicker.   × 32.   29 and 30. Sections near tip of leaf.   30. Close to tip.   × 75.   Adaxial surface very small.   *c.*, colourless parenchyma below upper epidermis.

in its leaves, and has a distinct adaxial epidermis of large thin-walled cells, though this only occupies a small portion of the surface, about one-sixth (Fig. 25).   The structure is like that of a very young leaf of *J. subnodulosus*, but with the adaxial epidermis present.

J. squarrosus.

The last type to be described shows some individual peculiarities. The leaves are spreading and bifacial.   In structure they show differences in different regions of the leaf.   The sheath (Fig. 26) has the usual structure.

It is somewhat thick in the middle with the bundles in more than one row, but all with the same orientation. In this part several strands, composed of fibres alone, occur along with the bundles. The lower portion of the limb (Fig. 27) has a typical bifacial structure with the adaxial surface channelled and concave in section. The bundles are in a broad U and are in one or two rows. Fig. 27 is a section of a leaf from a younger plant than Figs. 26 or 28, and has a smaller number of vascular bundles. This is the region of the leaf figured by Buchenau (5), Pl. II, Fig. 1. Passing upwards the concavity of the upper surface becomes less. The leaf in section is narrower and thicker; the bundles become closer together, the larger ones which are nearer the adaxial side form a more closed U (Fig. 28). This is the appearance in the middle region of the leaf. Nearer the tip the appearance shown in Fig. 29 is found. The adaxial surface is now shortened, and occupies not more than about a quarter of the apparent upper surface. This adaxial epidermis has below it colourless parenchyma cells that form a strip along the length of the leaf in this part; the bundles are in a horseshoe form. This structure persists to the tip (Fig. 30). This leaf, while retaining throughout its length the typical bifacial condition, demonstrates well the gradual suppression of the adaxial surface and the consequent approach to the unifacial state, which is accompanied by increasing curvature of the arc of bundles. In the distal portions (Fig. 29), the bundles near the adaxial side are placed at right angles to the median one, but they retain the same relation to the abaxial surface. Unfortunately seedlings of this plant and of *J. subnodulosus* were not obtainable for examination.

GENERAL CONSIDERATIONS.

The features described above all tend to support the views of Buchenau (5) and Goebel (11), that the unifacial leaf, which is such a marked feature of these plants, is derived from a bifacial type by the reduction and final suppression of the adaxial surface. Additional evidence is brought forward in several instances. In *J. maritimus*, for example, while the leaf is externally unifacial the arrangement of the bundles is distinctly dorsiventral. The stages by which such a condition may be reached are paralleled by the leaf of *J. squarrosus*. Here, in the distal parts, the adaxial surface is very much reduced, and the bundles are arranged in a U or horseshoe. In *J. maritimus* a further stage is attained; the adaxial surface is suppressed altogether, but the same bundle arrangement is maintained. *J. subnodulosus* shows a closer approach to radial symmetry. In the mature limb, with its large central cavity, this radial arrangement is almost or quite complete. In the development of the leaf, however, the origin of this form can be traced. The very young limb has

no central cavity, and at this time the arrangement of the bundles is just like that of *J. maritimus*—that is, clearly dorsiventral.

The most complete degree of radial symmetry is reached in *J. effusus*, where in the mature limb there is no structural indication at all of orientation. This plant has again a large central pith region. In this case the origin of the radially symmetrical unifacial leaf from a bifacial one, as the result of the suppression of the adaxial surface, is very clear. In addition to confirmation of the features described by Goebel (loc. cit.), the origin is well demonstrated by the succession of leaves formed by the seedling. The first leaves are completely bifacial, and very nearly identical in structure with those described in *J. bufonius*. Subsequent leaves show a marked decrease in the development of the adaxial surface, and finally its suppression, accompanied by an increase in the extent of the abaxial. Further, it has been shown that in the plants with unifacial leaves, the tip portion of the leaf has, in all cases, a dorsiventral arrangement of the vascular bundles. The leaf, at its first initiation, is dorsiventral even though the adaxial surface is completely suppressed, and the radial symmetry is assumed during its subsequent development. Now, at the same time, at the tip, it must be noticed that the pith region is very small or absent. As the central region enlarges, and the bundles become more crowded towards the periphery, their original arrangement becomes obscured, and a radially symmetrical one is approached. There seems a distinct correlation between radial symmetry and the production of a central cavity. Thus in *J. maritimus*, where the leaf never becomes hollow, the dorsiventral bundle arrangement is retained. In *J. subnodulosus* and *J. effusus* it is obscured or lost altogether in the mature state, though evident at the tip where no cavity is present, or in the very young leaf of the former. It may be mentioned here that the stellate pith tissue of *J. effusus* is apparently a secondary development. It is not present in the seedling plant, even in those leaves which have the adaxial epidermis suppressed altogether. Also in the allied plants, those which are regarded, on floral structures, as being the more primitive have hollow stems or leaves without pith, while the more advanced species have the stellate pith (cf. Buchenau (6)). The leaf of *J. squarrosus* differs from the others in the retention of a small area of the adaxial surface and in the elongated region of transition from the bifacial structure of the sheath to the almost unifacial one of the distal portion of the limb. The change of structure is attained in exactly the same way as in *J. effusus* or the other unifacial species (cf. Goebel (11), Fig. 293), but the reduction in extent of the adaxial surface is gradual, and is continued over a considerable portion of the leaf instead of occurring rather suddenly in a very short distance.

Whatever the mature form of leaf in *Juncus*, there seems no reason to doubt that it has been derived from a parallel-sided bifacial one. This is

the normal form of leaf in *J. bufonius* and many other species, and in the allied genus *Luzula.* This type is also produced as the first leaf in the seedling plant. All the other leaf types are derivable from this by a greater or less suppression of the adaxial surface, combined with the formation of a central cavity, and consequent peripheral arrangement of the vascular bundles.

The positions of the bundles in the leaf and their orientations are all to be explained as the result of these features of growth. If the cylindrical type with its bundles in a ring is to be regarded as a petiolar phyllode (Arber (1, 2, and 3, &c.)), then the bifacial leaf of *J. bufonius* must be regarded as being morphologically identical. The present study does not seem to afford any evidence either for or against the phyllode theory.

In the Juncaceae the bifacial leaf certainly seems to be the primitive form, and the unifacial to be derived from it (cf. Arber (3)).

SUMMARY.

1. The primitive leaf form is bifacial with the vascular bundles in an arc. This type is present in *J. bufonius* and other species.

2. *J. effusus* has a leaf with a perfectly radially symmetrical structure. This is produced as the result of suppression of the adaxial surface.

3. At the tip of the leaf in *J. effusus* the vascular bundles are arranged in a horseshoe open to the adaxial side.

4. In the seedling the first leaf has a bifacial structure just like that of *J. bufonius.* Subsequent leaves show a gradual suppression of the adaxial surface, and increase of the abaxial till the unifacial structure is reached.

5. In *J. maritimus*, which has a cylindrical solid leaf, the vascular bundles are arranged in a horseshoe and are distinctly dorsiventral.

6. In *J. subnodulosus* the mature leaf is almost radially symmetrical with a large central cavity. The young leaf which is solid, however, shows a bundle arrangement which is dorsiventral like that of *J. maritimus.* The tip also shows the dorsiventral structure.

7. *J. squarrosus* has a leaf bifacial in structure in the proximal portions of the limb, while in the distal portions the adaxial surface is very small, and the bundles are in a horseshoe.

8. The unifacial leaf type seems clearly derived from the bifacial by the suppression of the adaxial surface.

9. The positions occupied by the bundles are the result of this suppression of one surface and curvature of the other, combined with the production of a central cavity and the crowding of the bundles to the periphery.

The drawings illustrating this paper were all made with the aid of a Zeiss-Abbe camera lucida.

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Studies on the Sulphur Bacteria.

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With Plate XVI and five Figures in the Text.

INTRODUCTION.

THE present status of our knowledge of the sulphur bacteria is more or less comprehensively outlined by Skene,[1] Buder,[2] and recently by Waksman.[3] From these surveys it is apparent that many questions in regard to these curious forms still remain unanswered.

The nomenclature of the group is in a rather confused state—due to the fact that the names were given to the different forms when the nature of the organisms in question was imperfectly known. It seems doubtful, however, if any other nomenclature would be more satisfactory than the present one, as new discoveries might make a system of nomenclature obsolete overnight.

The forms loosely called sulphur bacteria were originally understood to be organisms able to use hydrogen sulphide as a source of energy. Later the forms that oxidized thiosulphate were added to the sulphur bacteria, and also the sulphate reducers. Organisms that oxidized free sulphur to sulphate were tacitly assumed to belong to this group also.

At present, therefore, it is difficult to say what is actually meant by the term sulphur bacteria. An absolute definition seems to be impossible as representatives of widely divergent morphological and physiological groups are included among them. Instead of centring our general description around the inorganic S-compound we will stress the autotrophic nature of the forms and describe the members of the group under names meant to

[1] M. Skene, New Phytologist, xiii. 1 (1914).
[2] J. Buder, Jahrb. wiss. Bot., lviii. 525 (1919).
[3] S. A. Waksman, Journ. Bact., vii. 231 (1922).

characterize their salient physiological features.   This scheme is not intended as an attempt to found a new system of classification.

THIOBACTERIA, oxidizing sulphur and sulphur compounds.

1. ENDOTHIOBACTERIA, storing the sulphur temporarily inside the cell.
    a. ENDOTHIORHODACEAE, or purple sulphur bacteria.
    b. ENDOTHIOLEUKACEAE, or colourless sulphur bacteria.
        (1) Filamentous forms, like *Beggiatoa, Thiothrix.*
        (2) Unicellular forms, like *Thiophysa, Thiovulum.*
2. ECTOTHIOBACTERIA, forming the sulphur outside the cells.
    a. ECTOTHIOLEUKACEAE (the corresponding purple forms are not known).   This group comprises
        (1) Forms oxidizing S-compounds to S, such as *Thiosulphate bacteria.*
        (2) Forms oxidizing both S and S-compounds, such as *Thiobacillus thioparus.*
3. THIOXIDANS group, oxidizing sulphur to sulphate.   *Thiobacillus thioxidans* of Waksman and Joffe.

There is one class of organisms, the *Athiorhodaceae*, purple bacteria without sulphur inside the cells, which is often included in the foregoing scheme.   The reason for this is the influence of Molisch's monograph on the subject.[1]   This author did not draw a sufficiently clear line between the Thiorhodaceous and Athiorhodaceous forms of the group.   This has resulted in confusion, and even now (Waksman, loc. cit. 235) leads to a misunderstanding of the true nature of these forms.   The deficiencies in the present nomenclature can be brought out by the following example.   Beyerinck[2] describes a bacillus that is able to oxidize S-compounds with the formation of free sulphur.   This sulphur is deposited outside the cell.   He calls this organism *Thiobacillus thioparus.*

In mud from Owen's Lake, California, a *Spirillum* was found which apparently is responsible for the deposits of free sulphur on the black sulphide mud.   In harmony with Beyerinck's nomenclature the name of this *Spirillum* has to be *Thiospirillum*.   In the literature, however, we find this name already used for two totally different forms, both endo-sulphur bacteria, one a colourless form, described by Omelianski,[3] the other a purple form, described by Cohn.[4]   No attempt will be made, however, to rename the organisms.

As the sulphur bacteria play an important rôle in the cycle of sulphur

[1] H. Molisch, Die Purpurbakterien. Jena, 1907.
[2] M. W. Beyerinck, Centralb. f. Bakt., 2. Abt., xi. 593 (1904).
[3] W. Omelianski, Centralb. f. Bakt., 2. Abt., xiv. 769.
[4] F. Cohn, Beitr. Biol. Pflanzen, i. 141 (1875).

in nature an attempt will be made to ascertain their true position in that cycle. The natural ecological community of these bacteria is a miniature cycle in itself, and will be called a SULPHURETUM.

The ecological study of these forms naturally centres around two main foci :

(1) The external milieu and (2) the internal milieu. The external milieu can either be physico-chemical or physiological. To the former group of factors belong such primary influences as light, oxygen, and salts ; to the latter group, all the organisms which constitute the sulphuretum. Under the factors of the internal milieu this paper will deal with the factors influencing the mechanism of sulphur formation.

Pure culture of an organism gives us certainty about a great many facts. It fails, however, to account for the particular place of the organism in an organic cycle, inasmuch as its occurrence is influenced and restricted by competition. Mass cultures, on the other hand, possess many latent features that can be brought out in the laboratory under controlled conditions.

Attempts at pure culture have been made during the course of this investigation. They have failed as far as the Thiorhodaceae were concerned. A few Athiorhodaceae and a thiosulphate reducer have been successfully isolated, however.

The author is fully aware of the many shortcomings of this paper, especially in regard to the failure of a pure culture method. Many of the results have, therefore, only a tentative value.

A. THE EXTERNAL MILIEU. PHYSICAL AND CHEMICAL FACTORS.
General Characterization.

1. The forms studied in this paper occur either in (alkaline) fresh water, brackish water, or brine, not more than twenty centimetres under the surface. They are either directly exposed to light or are covered with a thin layer of greenish débris (in fresh water), algae (*Enteromorpha* in brine), or $CaCO_3$ (in brine). The sulphuretum rests on a layer of black mud of variable thickness.

They are exceedingly common in the vicinity of Stanford University, where they occur in a great variety of forms. As they show specific relations to certain environmental factors some of these factors will be considered more in detail.

2. *Light.*

Winogradsky,[1] to whom we owe the classical account of the Endothiobacteria, states that light is a necessary factor for their development. He claims that this holds for the purple as well as for the colourless forms. While Buder and Skene have corroborated Winogradsky's data on the

[1] S. Winogradsky, Bot. Ztg., xlv, 488, 512, 528, 544, 568, 584, 600 (1887) ; Zur Morph. u. Physiol. der Schwefelbakt., Leipzig, 1888.

Thiorhodaceae, Keil [1] has proved that the development of *Beggiatoa*, on the contrary, is not influenced by light.

To Buder (loc. cit.) we owe an admirable ecological study of the purple bacteria. He states that the occurrence in shallow water is due to the lack of infra-red light in deeper water, which light is needed by the bacteria for their development. He bases this opinion on measurements of Aschkinass,[2] whose data indicate that the intensity of infra-red light of 800 $\mu\mu$ wave length is reduced to 10 per cent. by a water layer of about one metre.

This seems important because of Buder's idea (inspired by Engelmann) that the Thiorhodaceae are capable of photosynthesis, with a maximum activity in the infra-red. It seems, however, that the habitat of the sulphuretum is determined by other factors as well, as *Beggiatoa*, which is certainly not photosynthetic, arranges itself in much the same fashion.

Buder's chief idea in his monograph is to account for the colour and habitat of the Thiorhodaceae by the theory of complementary adaptation as proposed by Stahl,[3] Engelmann,[4] and Gaidukov [5] for their organisms.

As the absorption bands of the Thiorhodaceous spectrum alternate more or less with those of the chlorophyll, Buder is inclined to believe that this fact accounts for their natural occurrence under a layer of green aquatics. Buder's idea is illustrated in Text-fig. 1, representing two photo-microspectrograms taken of green and purple organisms of Owen's Lake, California. The left-hand spectrum is that of a single Euglenoid, possibly a *Eutreptia*; the right-hand spectrum, a colony of *Thiopolycoccus*. The Engelmann microspectroscope was used with a narrow slit on a Zeiss stand, objective F, tube length 180 mm. As a source of light the parallel beam from a carbon arc was used. Wratten Panchromatic film proved to be the most satisfactory. Exposure 90 seconds. A discussion of the properties of these spectra will be given elsewhere. It has to be stated, however, that the absorption spectra of the green top layer and the purple bacterial film are by no means antagonistic; the spectra are not complementary in the visible range.[6]

The bacteria would be able, under these conditions, to utilize the light transmitted by the top layer. The habitat as outlined by Buder was rarely encountered in the course of this work. The bacteria were sometimes hidden, it is true, under a layer of brownish débris or milky white $CaCO_3$.

[1] Beitr. zur Biol. der Pflanzen, xi. 312 (1912).

[2] F. Aschkinass, Wied. Ann. d. Phys. u. Chem., lv. 401 (1895).

[3] E. Stahl, Zur Biologie des Chlorophylls. Jena (1909).

[4] Th. W. Engelmann, Bot. Ztg., xli. 1 (1883).

[5] N. Gaidukov, Abh. Preuss. Akad. d. Wiss., Supplement (1902); Ber. d. Deutsch. Bot. Ges., xxiv. 1 (1906).

[6] L. B.-Becking, Journ. Gen. Physiol. (1925), in press (*Euglena*); Proc. Soc. Exp. Biol. and Med., xxii. 523 (1925) (Purple bacteria).

As a possible indication of active adaptation, however, it must be mentioned that the Thiorhodaceae from this station, *Rhabdochromatium* as well as *Chromatium*, are very pale pink, or lavender colour; while the freshwater forms are bright purple. In Owen's Lake the supernatant water about (5 centimetres deep) was coloured brown. The diatoms (Navicula) in this sulphuretum had become entirely green by chromatic adaptation [1] while the purple bacteria at this station had their ordinary brilliant colour.

On the other hand, there is no evidence to corroborate Miyoshi's view [2] that certain Thiorhodaceae do not need light. No development could

6750

5900
5700

5000
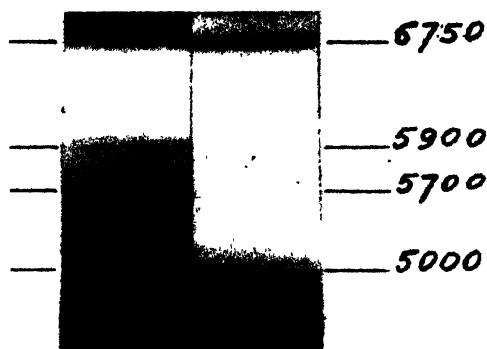
TEXT-FIG. 1.

be observed in the dark and a strong positive phototaxis was apparent in nearly all cases.

Every culture was carefully tested with an Engelmann microspectral objective. Although the accumulation on the infra-red (Buder) was quite evident in some cases (especially with *Chromatium*) frequent failures were noted, especially in the more saline media. Part of these failures may be due to personal factors; nevertheless, the persistent absence of accumulations at other parts of the spectrum shows that some factor was lacking in the experiments.

The quantity of the light needed by the Thiorhodaceae seems to be moderate. In the full Californian sunshine they move downwards in the mud. In shaded, very shallow creek beds, they sometimes occur entirely exposed. Such a case was observed in San Francisquito Creek, two miles west of Stanford University, where *Lamprocystis* created a remarkable colour effect. The growth in the laboratory is best at the north window; direct insolation kills them almost instantly (see, however, Athiorhodaceae).

Thiospirillum, notwithstanding its red colour, may occur in turbid

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[1] See, on chromatic adaptation in diatoms, Schorler, Arch. f. Hydrobiol., iii. 100 (1907).
[2] M. Miyoshi, Journ. Imp. Univ. Tokyo, x. 143 (1897).

water of culture jars down to a depth of 40 centimetres. In the laboratory, the cultures of *Lamprocystis* often show a growth at the surface of the water. *Thiopolycoccus* behaves similarly, while *Chromatium, Rhabdochromatium,* and *Thiopedia* seem to occupy an intermediate position. This distribution might be due to a great many other factors, however. Apart from the phototaxis of individual bacteria, the dependence on radiation cannot be proved by ecological considerations, as, in the absence of pure cultures, other organisms might influence the sulphur-containing members of the sulphuretum so as to simulate a positive effect (see under oxygen requirements).

3. *Hydrogen sulphide.*

Winogradsky has given the proof that the thiobacteria cannot live in water without H_2S. The careful experimentation of Keil on *Beggiatoa* has established this fact quantitatively as well as qualitatively.

In this paper it will be shown that, in all probability, H_2S is not in most cases the material oxidized by sulphur bacteria ; but that the hydrosulphide ion is their source of energy. As this hydrosulphide is derived from H_2S, however, we will consider briefly the occurrence and various sources of this gas in nature.

The ultimate source of H_2S in the sulphur cycle is the protein molecule.

Cystin   and   Cystein

$$
\begin{array}{ccc}
\mathrm{CH_2-S-S-CH_2} & & \mathrm{CH_2S-H} \\
| \qquad\qquad | & & | \\
\mathrm{CH.NH_2} \quad \mathrm{CH.NH_2} & & \mathrm{CH.NH_2} \\
| \qquad\qquad | & & | \\
\mathrm{COOH} \quad\; \mathrm{COOH} & & \mathrm{COOH}
\end{array}
$$
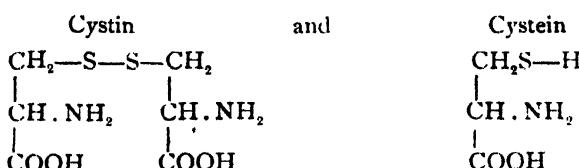
These are the two S-containing amino acids.

The amino acids can be broken down in various ways in the animal body. The older, positive scheme of cystein decomposition[1] seems to have little or no experimental support.[2] The so-called conjugated sulphuric acids appear as end products.

(2) In certain green plants thiocyanates appear which may be considered as end products of S-metabolism (Cruciferae).

(3) There is reason to believe that cystein, under certain conditions, gives off H_2S directly, changing to alanin.

$$
\begin{array}{ccc}
\mathrm{CH_2SH} & & \mathrm{CH_3} \\
| & & | \\
\mathrm{CH.NH_2 + H_2} & \longrightarrow & \mathrm{CH.NH_2 + H_2S} \\
| & & | \\
\mathrm{COOH} & & \mathrm{COOH}
\end{array}
$$
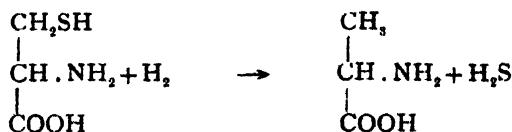
[1] See Abderhalden, in Oppenheimer's Handbuch, vol. i, p. 393 (1909).
[2] C. A. Schmidt and G. W. Clark, Journ. Biol. Chem., vol. liii, p. 193 (1923).

(4) Bacteria decompose cystein via such substances as

$$
\begin{array}{ccc}
\mathrm{CH_3} & \mathrm{CH_2SH} & \mathrm{CH_3} \\
| & | & | \\
\mathrm{CHSH} & \mathrm{COOH} & \mathrm{SH} \\
| & & \\
\mathrm{COOH} & &
\end{array}
$$

a Thiolactic Acid Thioglycolic Acid Methyl Mercaptan
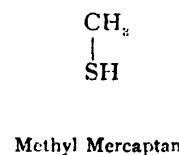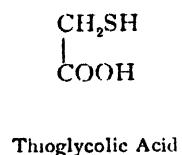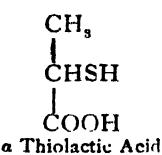
the mercaptan changing into sulphide or H_2S.

(5) Another source of H_2S is the anaerobic sulphate reduction.[1] This sulphate reduction must be considered a very important source of H_2S, especially in places where the organic content of the water is low.

(6) Many living cells, especially yeast, but also bacteria, are able to reduce sulphur to hydrogen sulphide.[2] However, this process is only important in nature where there is a supply of sulphur. We would, therefore, expect those forms to live in the same cycle with the exothiobacteria.

(7) Large amounts of H_2S may be liberated from iron sulphide in the black mud. This process can only take place when the acidity of the water is increased to $pH < 7$.

The first author, according to Beyerinck, who described the black mud in connexion with living organisms, was Braconnot,[3] who investigated this curious mineral in the sewers of Nancy. Beyerinck, in his study of anaerobic desulfuration,[4] describes the cyclic changes in this black mud as follows :

(1) $SO_4^{--} \rightarrow H_2S$ (anaerobic).
(2) $H_2S + Fe^{++} \rightarrow FeS$.
(2 a) $H_2S + Fe^{+++} \rightarrow FeS + S$.
(3) $FeS + H_2CO \rightarrow FeCO_2 + H_2S$.
(4) $H_2S \rightarrow S$.
(5) $S \rightarrow SO_4^-$.
(6) $S \rightarrow H_2S$.

This conclusion is similar to the one here presented, but there is one important difference. We shall see that alkaline waters have a considerable buffer value. It will take, therefore, a very high pressure of H_2CO_3 to lower the alkalinity sufficiently ($pH \sqrt{7}$) to cause a decomposition of the black mud. With a base concentration of 5×10^{-3} a H_2CO_3 concentration of 60 × that of the surface water is ordinarily required to lower the alkalinity sufficiently to cause a decomposition of black mud. In Searsville Lake, and other freshwater reservoirs near Stanford

[1] M. W. Beyerinck, Arch. des Sc. Exact. Nat. Harlem, xxix. 233 (1896).
[2] H. W. Redfield and F. C. Huckle, Journ. Am. Chem. Soc., xxvii. 3, 12 (1915).
[3] Ann. de Chim. et Phys., l. 213 (1832).
[4] Verzamelde Geschriften, iii. 102 (1921).

University, the concentration of the carbonic acid at the bottom is hardly ever higher than five times the surface concentration. Sea-water is alkaline and has a high buffer value; the hydrotroilite and the bottom of the Black Sea [1] can be formed, but not destroyed, the acidity apparently never becoming sufficiently high to decompose the mud. The black mud will, therefore, only become available through oxidation, or through a very marked rise in H^+ due to acid decomposition of carbohydrates (sludge, sewage, &c.). It is quite possible that, in a soft water, the amount of CO_2 generated by bottom bacteria may be sufficiently large to account for a hydrotroilite decomposition described by Beyerinck.

The buffer value of hard water may be so great, however, that FeS will only serve as a source of dissolved H_2S in rare occasions. The above enumeration shows the great number of natural sources of H_2S. This refutes the statement often met in the literature,[2] that the occurrence of H_2S in natural water is an indication of contamination with animal excreta.

The aqueous solution of hydrogen sulphide seems, therefore, to hold an important central position in the sulphur cycle. The equilibrium in such a solution will be discussed in the third section of this paper. The origin of the H_2S in the sulphuretum depends on the situation in waters containing sulphite waste of paper mills, for instance. The source of H_2S will be different from those waters in which the H_2S is entirely derived from organic remains (sewage, plants, plankton). However, I feel inclined by experience to believe that sulphate reduction is the chief source of H_2S and its salts in natural waters.   ·

4. *The concentration of the hydrogen ions* in the sulphuretum is consistently low; the S bacteria occur in hard waters only. In more than thirty different cases tested with indicator dyes (chiefly cresole red), the pH ranged from 7·6 to 8·6. This is a similar range to that found by Atkins [3] for hard waters. It was found that the pH decreased considerably in waters where putrefaction liberated CO_2 and organic acids. We shall have occasion to revert to this problem later. The high alkalinity of water is due to the large amount of calcium and magnesium present. The water possesses a considerable buffer value due to its carbonate and bicarbonate content. The buffer value of fresh lake water (Searsville Lake, a reservoir near Stanford University) was determined in the following way:

50 c.c. of lake water were titrated with 0·01 N/HCl. The average of twelve experiments is given in the table. The H^+ is expressed as pH. The titrations were performed with different standard indicator dyes.

[1] Nature, xlviii. 323 (1893).
[2] L. S. Thompson, Journ. Med. Res., xlii. 383 (1921).
[3] W. R. G. Atkins, Trans. Far. Soc., xviii. 310 (1923).

pH.	c.c.	0·01 N acid.
8·2 ⎫ 7·0 ⎬ 6·0 ⎭	2·24 9·93	5 ⎫ 3·03 4 ⎬ 3 ⎭ 0·84
5·0 ⎫	10·29	

This shows the presence of a very high buffer value between pH = 7 and pH = 5.

Before we draw any conclusions as to the ecological importance of the pH and high buffer values, it might be well to follow Johnston[1] in his considerations of the rôle of the carbon dioxide in natural waters.

The carbon dioxide in the atmosphere is in equilibrium with a concentration of carbonic acid in the water.

$$(H_2CO_3) = C \cdot P_{CO_2} \quad \dots\dots\dots\dots\dots\dots\dots(1)$$

This carbonic acid is dissociated.

$$K_1 (H_2\dot{C}O_3) = (H^+)(HCO_3^-) \quad \dots\dots\dots \dots\dots(2)$$
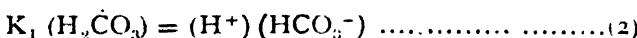
in which $K_1 = 3\cdot4 \times 10^{-7}$.

The bicarbonate ion again dissociates
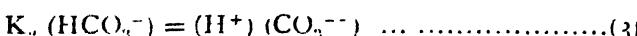
$$K_2 (HCO_3^-) = (H^+)(CO_3^{--}) \quad \dots \dots\dots\dots\dots(3)$$
$$K_2 = 4\cdot6 \times 10^{-11}.$$

Furthermore, the ionic product of water

$$K_w = 8 \times 10^{-15} = (H^+)(OH^-)\dots\dots\dots\dots\dots\dots(4)$$

and evidently

$$(B^+)+(H^+) = (OH^-)+(HCO_3^-)+2(CO_3^-) \quad \dots\dots(5)$$
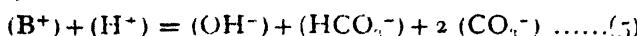
(total base).

It is possible to solve these equations and express the concentration of carbonic acid in total base and hydrogen ion. It is easily shown that

$$(H_2CO_3) = \frac{\{(B^+)+(H^+)\}(H^+)^2 - K_w(H^+)}{K_1(H^+) + 2K_1 K_2} \quad \dots\dots(6)$$

This equation allows us to determine H_2CO_3 when B^+ and H^+ are known, or vice versa. B^+ can be determined by titration; H^+ by indicator methods.

From equation (6) it can also be seen that 'titratable' B^+ and 'real' H^+ acidity may even vary in inverse directions, if H_2CO_3 remains the same. This is true for strong saline solutions, such as brines, in which B^+ is very high, but H^+, by being low, causes a normal CO_2 content (H_2CO_3) of the water. Later in the paper the results of equation (6) will be applied.

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[1] John Johnston, Journ. Am. Chem. Soc., xxxviii. 947 (1916); J. Johnston and E. D. Williamson, Journ. of Geology, xxiv. 729 (1916).

Attention must be called to equations (2) and (3)—

$$3.4 \times 10^{-7} \ (H_2CO_3) = (H^+) (HCO_3^-).$$
$$4.6 \times 10^{-11} \ (HCO_3^-) = (H^+) (CO_3^{--}).$$
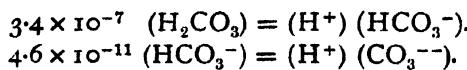
The latter can be written :

$$\frac{(HCO_3^-)}{(CO_3^{--})} = 2.2 \times 10^{10} \ (H^+).$$

From this equation we can calculate the relative proportion of bicarbonate and carbonate ion in a solution of known hydrogen-ion concentration. A change in the H^+ due to organisms may be caused either by

(1) CO_2 assimilation, changing

$$2 \, HCO_3^- \text{ to } CO_3^{--} + H_2O + CO_2, \text{ or}$$
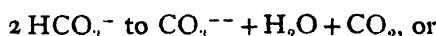
(2) respiration, changing

$$CO_3^{--} + H_2O + CO_2 \text{ to } 2 \, HCO_3^-.$$
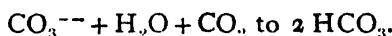
Such changes in a *pure culture* of sulphur bacteria might decide the question as to their contested power of CO_2 assimilation (Keil, loc. cit. on p. 637). The presence of large amounts of photosynthetic algae in the sulphuretum, however, makes such a determination untrustworthy.

The carbonate and bicarbonate ion in the water undoubtedly are to be considered as the chief factors in determining its alkalinity. We have, however, to mention the H_2S as another influential factor, and in sea-water and brine the so-called salt error. Surface water, digested at 30° C. for two months, increased its hydrogen ion from pH 8·2 to pH 7·6. Atkins (loc. cit., p. 311) mentions that seawater may increase in H^+ by decaying material to a pH of 7. It is evident that the buffer action curbs any further action on the hydrogen-ion concentration. The fact that the normal sulphureta occur in hard waters of a pH around 8 shows that there is little bacterial action by ordinary putrefactive bacteria. The thiobacteria themselves, being autotrophic, will probably use the bicarbonate ion to obtain CO_2. But, as they are never found even in neutral water,[1] the amount of normal bacterial action, and therefore the *amount of organic matter present, must be small*. Beyerinck[2] states that ' die eigentliche an Schwefelwasserstoff adaptierte Fauna und Flora ist auf Brackwasser und Meerwasser angewiesen, und wir finden in unseren süssen Gewässern davon nur relativ wenige Repräsentanten '. This statement is certainly true for the soft waters of Holland in which true sulphureta are rare. But there is hardly a mountain stream or a watering trough on the San Francisco peninsula that does not contain such a flora.

[1] G. West, this Journal, xxvii. 113 (1913), describes the occurrence of an endothiobacterium in a *Sphagnum* bog. Bog water is often quite acid (pH about 5). Winogradsky's Langenbrücker water was, of course, quite alkaline. See also G. Hannevart, Acad. Roy. Belg. Sc., xii. 600 (1920).

[2] Centr. f. Bakt., I. i. 49, 104 (1895).

5. *Salinity.*

The sulphuretum seems to be independent of the salt concentration. Since Warming described his marine forms[1] the accounts of purple bacteria from sea-water have been numerous. Hitherto. however, no accounts have been published of thiobacteria from brine. The Redwood salterns contain, in certain sloughs, a very remarkable sulphuretum. This sulphuretum is maintained throughout the season as a pinkish film between the lime layer and the black mud. The concentration of the salt amounts from 7 to 8 per cent.

Almost identical forms occur in fresh water. The salt concentration, apart from the presence of enough Ca and Mg to cause a certain alkalinity, does not seem to affect the bacteria much. As a total analysis of the different waters is outside the scope of this investigation, a short method was devised [2] to determine roughly the amount of dissolved electrolyte by conductivity.

The electrolyte normality of three different sulphureta was determined. In case of the strong brine the aerometer reading was taken as a confirmatory measurement.

Station.	*Resistance in Ohm..*	Λ.	*Principal Forms.*
Searsville Reservoir	980	$1\cdot6 \times 10^{-2}$	*Chromatium, Lamprocystis, Thiospirillum. Beggiatoa.*
Owen's Lake	48	$2\cdot2 \times 10^{-1}$	*Chromatium. Rhabdochromatium, Thiopolycoccus, Thiopedia, Beggiatoa.*
Redwood City Brine	9	$1\cdot15$	*Chromatium, Rhabdochromatium, Amoebobacter, Beggiatoa.*
$0\cdot1$ N/KCl	104	$1\cdot2 \times 10^{-1}$	

The brine, calculated as NaCl, would be :

$$1\cdot15 \times 58\cdot5 \text{ per cent. strong } = 6\cdot7 \text{ per cent.}$$
$$\text{Aerometer reading (for NaCl)} = 7\cdot5 \text{ ,, ,,}$$

As the first number is decidedly too low ($CaSO_4$, $Ca(HCO_3)_2$, &c., present), there is a satisfactory agreement between the two methods. Mitchell[3] determined the total solids in Searsville water as 377 parts per million in August 1907. 1923–4 was a particularly dry season. The total titratable base in Searsville (March 1924) amounted to $0\cdot02$ N. This checks with the conductivity measurement, but is decidedly high when compared with Mitchell's data. Assuming the metals to be chiefly Ca and Mg, the anions chiefly HCO_3^- and SO_4^{--}, we would expect an 8 per cent.

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[1] E. Warming, Vidensk. Med. Natur. Forsk. Kjøbenhavn. 20 (1875).
[2] Will be described elsewhere.
[3] J. P. Mitchell, A Study of the Normal Constituents of the Potable Waters of the San Francisco Peninsula. Stanford University, series No. 3 (1910).

solution to be about normal. 10^{-2} N would be about 800 parts per million. However, Mitchell obtained values as high as 843 parts per million from West Union Creek, one of the branches of San Francisquito, in the immediate neighbourhood of Searsville Lake, in July 1907.   The waters in this region are very hard.

From the above considerations it is apparent that the salt concentration is of little importance to the sulphuretum.   The chloride ion in Searsville, for instance, is present in a concentration of 7 parts per million ; in the brine several thousand parts per million.

The only ions that do not seem to change very much in concentration in the external habitats are the calcium ion and the magnesium ion. They determine chiefly the hydrogen-ion concentration.   Of great biological importance is also the iron.

In the hard waters studied it is insoluble, and occurs in suspension as hydroxy-carbonate ; one of the chief causes of turbid water.   This hydroxy-carbonate will lose its CO_2 at the air, and the pool may become covered with an iridescent film of iron hydroxide.   At the bottom of the lake or pond the iron is present either as red oxide, or, more frequently, as black hydrotroilite $(FeS)_x (H_2O)y$.   This hydrotroilite is a fine granular mass of low solubility ($42 \cdot 10^{-5}$ molar) at the neutral point.   At a lower H^+ concentration it is almost entirely insoluble.[1]   Hydrotroilite can only exist at a low oxygen tension.   When exposed to the air in a Petri dish, only the part covered with a slide will remain black, while the mud becomes grey at the exposed places.   Around the slide a red ring of iron oxide develops, secreted by iron bacteria.

In the freshwater mud only these bacteria could be observed.   Together with the common *Leptothrix ochracea*, the curious twisted sheaths of *Gallionella* formed the red ore.   During the oxidation of the black mud a smell of H_2S could be observed in the exposed parts of the mud, the H^+ increased from $10^{-7 \cdot 5}$ (black mud) to $10^{-6 \cdot 4}$ (grey mud).   The oxidizable nature of the suspended hydroxyde and hydroxy-carbonate could also be demonstrated by the presence of a chrysomonad flagellate in the freshwater plankton.   Especially one, *Trachelomonas*, surrounded by a sheath of bright brown iron oxide, is very conspicuous in the black mud.   Its CO_2 is derived from bicarbonate, but the oxygen given off during photosynthesis causes a local oxidation of the ferrous iron, suspended in the surrounding water. In being oxidized locally to ferric iron, which is even less soluble at the low H^+, the iron oxide precipitates around the photosynthetic organism, forming a brown sheath.

The rôle of the iron in natural waters, therefore, is more or less of the nature of a ' mineral reserve '.   In hard waters it forms the chief storehouse of sulphide, the surrounding supernatant liquid being saturated with nearly

[1] Atkins, loc. cit., p. 313.

insoluble, nearly unionized calcium and magnesium hydrosulphide, and, to a lower degree, sulphide.[1]

Skene (loc. cit.) and Keil found sulphates necessary for the growth of the S bacteria. The amount of sulphates in the fresh water is very small ; in the brine, however, there is much sulphate. There is evidence to show that sulphate chiefly promotes the development of the 'desulphuricans' group, giving rise to sulphides.

Nitrates and nitrites are present only in small traces in the water investigated. This was also the case in the Langenbrücker water used by Sergius Winogradsky.[2]

6. *Organic matter.*

The amount of dissolved organic matter is negligible in case of the brine sulphuretum. A filtered brine was titrated with permanganate in the cold and also at 100° C. for thirty minutes.[3] No difference was found in the 'oxygen consumed'.

This would seem to indicate that the amount of organic matter in solution is negligible. In other words, the H_2S probably originated from sulphate or sulphur rather than from putrefaction. Sulphate reducers, however, are not autotrophic. A small amount of dissolved organic matter has therefore escaped analysis. · Some authors contest the autotrophic nature of the thiobacteria on the ground that they live in 'polysaprobic' surroundings.[4] Apart from the elastic use of limnological terms such as 'polysaprobic' and the personal disagreeable associations caused by the ill-smelling black mud this idea is unjustifiable. It has been shown by Skene and Keil that organic material is without influence on the sulphur bacteria. Indirectly, however, it might be of great influence.

The addition of organic materials to culture jars gave unexpected results. Addition of small amounts (0·1 per cent.) of peptone and glucose caused an abundant reappearance of *Lamprocystis roseo-persicina* after a prolonged absence from the visible plankton community. The beneficial effect showed in about ten days at 18° C.

Addition of small amounts (0·05 per cent.) of soluble starch, however, caused a complete destruction of *Lamprocystis*.

The explanation of the beneficial effect of organic material lies in the complexity of the S-cycle. Sulphur, in our cultures, was present only as $CaSO_4$ 2 Aq (Winogradsky's method). In order to create a suitable environment for thiobacteria the sulphate has to be reduced. The sulphate

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[1] O. Weigel, Ztschr. f. Phys. Chem., 58, 294 (1907); L. Branner u. G. Zawadski, ibid., 65, 136 (1909); 67, 454 (1910); E. Fischer's Method for detecting H_2S, Ber. d. d. Chem. Ges , 16, 2234 (1883).

[2] Winogradsky, loc. cit.

[3] Standard Methods of Water Analysis, 4th edition, Boston (1920), p. 26.

[4] R. Lauterborn, Verh. Nat. Med. Ver. Heidelberg, xiii. 395 (1915).

reducer, being heterotrophic, required organic carbon. Only when a suitable concentration of H_2S (and dissociation products) had accumulated could the *Lamprocystis* start its development.

7. *Oxygen.*

The oxygen requirements of the sulphuretum must necessarily be very low. There cannot be free oxygen in immediate contact with the black mud.[1] Notwithstanding Engelmann's and Buder's considerations (see Section III) the oxygen development of the S bacteria is not yet proved. If such a development took place it should be possible that a Fe_2O_3 formation, similar to that in *Trachelomonas*, would be the result. As a matter of fact, such sheaths were never observed. However, around the colonies a brick-red débris can often be seen, which gives iron reactions with ferricyanide ions.

It is difficult to prove, however, that the presence of this oxide is due to the green algae, the green bacteria, or to the Thiorhodaceae. A shift in the bicarbonate equilibrium (increase in pH) was observed in the sulphuretum when illuminated. However, this may also be due to the presence of other autotrophonts. Only a pure culture will finally decide the matter. Although the sulphur bacteria prefer a certain depth, the culture of these forms under aerobic conditions has succeeded. A loopful of *Lamprocystis* was transferred to a flask containing:

Tap water	1,000	$CaSO_4$	10
Glucose	1	Peptone	1

After ten days a dense purple film covered the surface of the water, consisting almost entirely of *Lamprocystis.* This transfer method was repeated, without, however, purifying the *Lamprocystis* from the accompanying blue-green and green algae.

The *Lamprocystis* stayed in the upper 2 mm. of the fluid throughout the entire observation period (three months). The oxygen requirement of *Thiopedia* and *Thiopolycoccus* seemed to be of a similar nature. They occupied the upper centimetre of the liquid. Other Thiorhodaceae occur at different levels. *Lamprocystis* in another case lived at a depth of 10 cm., *Rhabdochromatium* 15–20 cm., *Chromatium* 5 cm., in another case 20 cm. *Thiospirillum* reached a depth of over 40 cm. It cannot only be the light which determines this curious distribution. It is probable that the presence of some other member of the sulphur cycle determines the position of the Thiorhodaceae in the culture jars (sulphate reduction, sulphur reduction).

The relation between oxygen and the energy relations will be treated in Section III.

8. Summarizing the results obtained for the inorganic external milieu we can say that, on the whole, the endothiobacteria require a certain

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[1] G. Romyn, Water, Bodem, Lucht, iii. 208 (1913).

amount of light, an alkaline water in equilibrium with a certain amount of H_2S. They are remarkably indifferent towards salinity and oxygen. The temperature was not considered in this section. as the forms were observed throughout the year in equal abundance, wherever water was available. This, however, does not mean that thiobacteria can develop in a very wide range of temperatures. The evenness of the climate in California is probably the cause of their abundance in summer and winter.

B. The External Milieu. Members of the Sulphuretum.

The sulphureta in brine as well as in brackish water or fresh water possess an analogous structure.

It is a community in which similar organisms occur in a small self-supporting cycle. It is impossible to understand the proper function of all different members of the group. About some organisms we may have an inkling as to their place in the cycle.

Without venturing, as yet, to ascribe a certain rôle to the organisms, we will deal with them separately.

I. *Thiobacteria.*

   (*a*) Endothioleukaceae.

      Beggiatoa (Pl. XVI, Figs. 1-4). Without any doubt the commonest form occurred in fresh water as well as in brine, various species with filaments of different thickness, motility, and length. *Beggiatoa*, unlike the other thiobacteria, is sensitive to temperature, and shows a decided optimal frequency in the summer. The cells are often apparently entirely filled with S droplets.

      Thiothrix (Pl. XVI, Fig. 10). In watering troughs fed from Searsville main pipe-line. Aerobic, influenced by temperature. S droplets often wider than the filament.

   (*b*) Endothiorhodaceae.

      Coccaceae.

      Lamprocystis roseo-persicina (Pl. XVI, Figs. 8-9). A common freshwater form. Occurs in the well-known irregular colonies of dark purple cells, each with one large sulphur droplet, and embedded in a mucilaginous sheath. Mucilaginous strands often connect the daughter colonies, as was clearly brought out by Indian ink. A swimming protozoon would often gather a whole mass of *Lamprocystis* around its body, and, caught in the mucilaginous strands, would actually be immobilized.

The *Lamprocystis* seems to be not so indifferent to oxygen as the other purple forms: it was cultivated at the surface layer of the culture fluid for several weeks. The carbon bisulphide extract of the dried bacteria yielded a fragrant substance (anethol-like smell).

Amoebobacter was encountered in several cases in fresh water and brine. No new facts can be added to Winogradsky's description.

Thiopedia, a tetrapediococcus, occurred in the Owen's Lake sulphuretum (Pl. XVI, Fig. 7). The cells often contain two to three sulphur droplets.

Thiopolycoccus (Pl. XVI, Fig. 15) formed the chief purple vegetation in Owen's Lake. Large (80 μ) globular colonies, dark purple in colour, intermingled with colonies that appear black in transmitted light, but are a brilliant purple in the dark field. These dark colonies contain sulphur. In the others no trace of sulphur could be found. As the S-containing colonies are only few in number, the coccus creates the impression of an Athiorhodaceous form, like the forms described by Molisch.

Thiospirillum jenense, Cohn (Pl. XVI, Figs. 5, 6). ' Bacterial mammoth.' To 75 μ long, brilliantly red, extremely motile. Occurs at greatest depth of all the forms described (40 cm.). It shows the coalescing of the droplets by heat or picric acid (Pl. XVI, Fig. 6). Loeffler's stain brings out a lophotrichous bundle of cilia at one end (Pl. XVI, Fig. 5).

Chromatium. The most widespread form. Was only absent in one *Lamprocystis* culture. The genus ranges from large (16 μ) pale pink forms to small brilliantly coloured bacteria (2 μ). The pale pink brine form is only feebly phototactic, the freshwater form much more so. Loeffler's stain failed to bring out the cilia in many cases. This may be due to a mucilaginous secretion which becomes apparent as soon as the forms cease to swarm (Pl. XVI, Figs. 12–14).

Rhabdochromatium was absent from the fresh water. It often suggests a long row of *Chromatium* forms with a total length of nearly 40 μ. It was the chief occupant of the brine sulphuretum for several months.

To the naked eye the coloured forms show a great range of variation. *Lamprocystis* and *Thiopolycoccus* appear as a deep purple film, the former with a bluish tinge, on which darker and lighter spots can be detected, as the size of the colony often exceeds the limits of visibility. *Amoebobacter* has a rather vulgar pink colour, and never formed a large film in the cases observed. *Chromatium* is decidedly less bluish and can be detected by its swarmers, which blur the clear outline of the bacterial film, often occurring in a cloudy suspension. *Rhabdochromatium* formed rings about 10 cm. under the surface of the water.

Arranging the purple forms in the order of their occurrence, we find that *Chromatium* is the commonest; a close second is *Lamprocystis*. Decidedly less common are *Amoebobacter* and *Rhabdochromatium*, while *Thiospirillum*, *Thiopedia*, and *Thiopolycoccus* were observed in only one case.

II. Protozoa, feeding on the S and other bacteria, were a common feature of the sulphuretum. Only the *Paramoecium* group of the ciliates, containing reserve glycogen, was observed   This is a significant fact, as

Paramoecium is able to lead anaerobic life [1] (intramolecular respiration). *Amoeba proteus* and *limax* are common features in the fresh water, while the amoeboid forms were represented in the brine by a small lobed form, always filled with a number of partly digested *Chromatium*.

The ciliates in the brine showed some unusual features. They belonged to the large, *Paramoecium*-like type. No attempt was made to classify them. The thiobacteria seem to be the chief food of the ciliate and amoeboid protozoa.

Mr. Florencio Talavera,[2] in my laboratory, has studied the fate of ground rhombic sulphur in ciliate and amoeboid cells. As representative species *Paramoecium bursaria* and *Amoeba proteus* were used. The *Amoeba* incorporated the sulphur granules in its vacuole. There was no apparent change in these granules for forty-eight hours. In the *Paramoecium*, however, the sulphur granules became invisible after twelve hours. A large number of crystals appeared (Pl. XVI, Fig. 16) which gave the reactions on $CaSO_4$. Schewiakoff has claimed the crystals in *Paramoecium* to be phosphate. In addition to this it is known that, in certain instances, *Paramoecium* is able to deposit gypsum crystals inside the cell. The excretion of these crystals seems to be very difficult. Both ciliate and amoeboid protozoa seem to prefer sulphur bacteria as a food to any other organism in the sulphuretum. Only in *Paramoecium* the fate of the sulphur could be followed to SO_4^{--} ion.

A very striking member of the sulphuretum, in brine as well as in fresh water, is a large Spirochaete, about 200 μ long. It often occurs in great abundance and was never absent from any culture examined, although it is not common in ordinary lake or creek mud around Stanford. *Euglena* of various types and also *Phacus* are the chief representatives of the flagellates in the sulphuretum. These forms are adapted to low oxygen tension, and are also supposed to be an indication of contamination with organic matter.

However, after filtration of the culture through a Chamberland candle, the permanganate method for oxygen consumed [3] showed, in the case of a sample from San Francisquito Creek, less than 1 mg. dissolved organic carbon per litre (on a basis of $C_6H_{10}O_5$). The amount of dissolved organic matter was, in this case, low. The brine-inhabiting counterpart of the freshwater *Euglena* was a curious Euglenoid form, to 100 μ long, with numerous green chromatophores, two flagellae, and a red eye-spot. The organism was extremely plastic, changing its shape like a veritable contortionist. Dr. Ch. A. Kofoid, from the Department of Zoology, University of California, had the kindness to identify this form as *Eutreptia viridis*,

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[1] H. Wallengren, Ztschr. Allg. Physiol., i. 67, 1902.
[2] Master of Science Thesis. Stanford, 1922.
[3] Standard Methods for the Examination of Water and Sewage. Journ. Publ. Health Ass. Boston, 1920, p. 25.

Perty, var. *schizo-chlora*, Entz, originally described by Dr. Gésa Entz from the brine pools in Számosfalva, Hungary.[1]   Redwood City is, as far as the author is aware, the second brine locality of this curious flagellate.

III. *Nematodes* were always present. The literature accounts for them as polysaprobic and with anaerobic tendencies.[2]   The scavenger nature of the free living forms is known.   No attempt was made at classification.

IV. *Phormidium* and *Oscillatoria* were the Cyanophyceae represented in the fresh water, but never in such abundance as to become the chief members of the community.   In Owen's Lake a slender *Spirulina* (*S. Meneghiniana*?) in the strong brine and *Spirulina subsalsa* were the only blue-green forms observed.   Diatoms were abundant at all stations.   Only simple *Protococcus*-like forms represented the green algae in this community, thus showing the sulphuretum to be adapted to a low nitrate content (blue-greens can live with but little nitrate in the culture medium).   Occasionally observed were :

Trachelomonas, spec. div.	(two cases)
Cosmarium	(one case)
Pediastrum	(one case)
Oedogonium	(one case)

V. *Green Bacteria*. Van Tieghem[3] was the first to observe green cocci in the sulphuretum.   Since 1880 a large number of these forms have been described, and their rôle in the sulphuretum has been the source of much speculation (Winogradsky, loc. cit.).   The first real advance in our knowledge of the forms was made when Metzner[4] gave the spectrum of their pigments, proving its dissimilarity with that of chlorophyll.

The green bacteria are no constant companions of the sulphuretum, as many authors assume.   The author has never been able to find them in brine.   In the brackish water of Owen's Lake, however, a tiny green coccus was present, while in a peptone *Lamprocystis* culture a dark green *Streptococcus* appeared.   This *Streptococcus* could be grown on 3 per cent. peptone agar, was aerobic, but was not obtained in pure culture.   It consisted of eight to eighteen individual cocci 2 to $2\frac{1}{2} \mu$ long and about 2μ wide.   The forms occurred chiefly at the surface of the mass cultures, but a few weeks after their appearance they began to form a blackish-green precipitate on the bottom of the jar.   From Ewart's[5] description it appears to fit almost exactly to the name of *Streptococcus varians*, Ewart.   Winogradsky and others see in the green bacteria symbionts which provide the thiobacteria with the necessary oxygen.   There are three reasons against this assump-

[1] G. Entz, Természetrajzi Fuzetek, vol. vii, 139, 1883.
[2] Hermann Jordan, Vergleichende Physiologie wirbelloser Tiere, i, Ernährung, 176 (1912).
[3] Bull. Soc. Bot. de France, xxvii. 174 (1880).
[4] P. Metzner, Ber. d. deutsch. Bot., xl. 115, 1922, where a survey of the literature can be found.
[5] A. J. Ewart, Journ. Linn. Soc., xxxiii. 123 (1897).

tion: (1) They are not constant companions of the sulphuretum; (2) the amount of O_2 excreted by them must be small as compared with the amount excreted by the Cyanophyceae and Euglenids present; (3) the oxygen excreted is bound by the sulphide before it can reach the thiobacteria.

VI. *Athiorhodaceae.* After a survey of the literature, Buder (l. c., p. 535), in his monograph on the purple bacteria, arrives at the following conclusion in regard to the Athiorhodaceae: ' So ist mir doch weder aus der Literatur noch aus eigener Anschauung ein ähnlich auffallendes Vorkommen in der freien Natur bekannt geworden, wie bei den roten Schwefelbakterien. Immer waren es Aufgüsse und ähnliche Herrichtungen, in denen ihre Entwicklung eine solche Uppigkeit erreichte, daß sie dieselbe schon dem bloßen Auge durch ihre Farbe verrieten.'

Mr. Hilarion Silayan, who was working in the Botanical Laboratory of Stanford University on some problems in rice-culture, noticed that the water on his culture jars and tanks became wine-red about ten days after the rice was put in. Examination of the water revealed the presence of a slender dark-red bacillus, apparently identical with Molisch's *Rhodobacillus palustris.*[1]

The individual bacilli were about 6–10 μ long, combined to small, irregular colonies. At the same time an Athiorhodaceous form was discovered in a mass of rotting *Fucus*, on the beach near Moss Beach. This form proved to be a larger *Rhodococcus.* A third, a small *Rhodococcus* form, occurred at the bottom of a tall graduated vessel used for starch culture of *Clostridium butyricum.*

In a *Lamprocystis sulphuretum* (from a watering-trough, Palo Alto Stock Farm) a small purple *Diplococcus* was found. All those forms were obtained in pure culture by the agar-shake method of Molisch on the following nutrient medium :

Tap water	1,000 grm.
Agar	18 grm.
Peptone	5 grm.
Soluble starch	5 grm. [2]

One drop of the material was diluted with 10 c.c. sterile tap water, and this shaken with the nutrient medium while still warm. The medium was then poured into sterile tubes. The red colonies appeared after five to nine days, and were transferred by breaking the tube and dissecting the contents with a sterile knife. A second shake was started, and proved to be pure in most of the cases. The *Rhodobacillus* developed all over the tube,

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[1] See Die Purpurbakterien, p. 14, Pls. I, II.

[2] Soluble starch instead of glycerine or dextrin as recommended by Molisch. The starch was prepared according to a method developed by Dr. O. L. Sponsler in this laboratory. Potato starch was ground in a pebble mill for seventy-two hours. 75–80 per cent. goes into solution in *cold water.*

apparently more or less indifferent to oxygen. This was also the case with the *Rhodococcus* from *Fucus*, which stood the transfer from its saline medium quite well. The small *Rhodococcus* from starch and the *Diplococcus* from the freshwater sulphuretum, however, proved to be decidedly micro-aerophilous. The cultures developed only at a depth varying from 40 to 80 mm. The bacteria developed only in the light. Without publishing the lengthy laboratory protocol, the results can be briefly summarized as follows :

Five sets of agar shakes, pure culture transfers, were started and placed in north windows, in strong artificial light, behind a red screen (transmitting red and infra-red), behind a blue-green screen (transmitting blue, green, and yellow), and in total darkness. The cultures in the north window and behind the red screen all developed normal, brilliantly red colonies. The cultures in strong artificial light also developed red colonies, of much smaller diameter, however. The cultures in the dark were negative throughout. Except for one contamination, no bacteria showed in these tubes. The cultures behind the blue-green screen did very poorly. Only the large *Rhodococcus* developed a set of pale-pink colonies. This is in perfect harmony with the findings of Skene (loc. cit., p. 14) for the Rhodothiobacteria.

This experiment proves that (with the possible exception of the large *Rhodococcus*) we actually had isolated representatives of the Athiorhodaceae, sensitive not only to the infra-red (as proved by Engelmann [1]), but also developing chiefly in light of long wave-length. Due to the presence of soluble starch in the nutrient medium, there was a copious development of *Clostridium* during the first few days of the experiment. This development could be brought to a standstill by strong illumination. In such a mass shake-culture it was found that prolonged illumination, however, will also kill the Athiorhodaceae, leaving only the green algae. From a pure culture of *Rhodobacillus* both bacteriochlorin and bacterio-erythrin could be isolated by Molisch's method (using strictly absolute alcohol, however ; see Skene, loc. cit., p. 8).

The CS_2 bacterio-erythrin solution quickly fades in the light. The faded solution on evaporation yields beautifully irridescent pinakoids, melting at about 180° C. The amount of bacteriochlorin seems to vary with the age of the culture. In young cultures there seems to be little, in old cultures we find considerably more (as shown by alcoholic extract). Old cultures become reddish-brown instead of purple.

Pure cultures of *Rhodobacillus* were 'fed' with H_2S water every day during two weeks. Although they continued to grow, no deposit of sulphur could be observed, either inside or outside the cells. *Rhodobacillus* will stand a concentrated solution of H_2S for several days without apparent injury.

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[1] Bot. Ztg., p. 661 (1888).

The medium of *Rhodobacillus* was acid (pH 6·2) due to the decomposition of the starch. H_2S treatment (Winogradsky's dilute solution) after neutralization with lime, however, killed the bacteria.

Therefore, although Thiorhodaceae and Athiorhodaceae have similar pigments, and behave similarly in the light, and probably develop with the aid of similar light, they cannot at present be considered as belonging to the same physiological group.

It is granted that the experiments described above are not conclusive, and that many more lines of work suggest themselves. Also that *Thiopolycoccus* (see above) seemingly occurs in Athiorhodaceous and Thiorhodaceous form side by side. As no further experiments were carried on, however, no sufficient evidence was obtained to look upon the purple bacteria as a homogeneous group.

It seems, however, that Buder's assertion is unwarranted. Athiorhodaceae do occur in a natural medium, *provided* that this medium contains assimilable carbohydrate (germinating rice, pentosan in *Fucus*). It would be interesting to inquire about their occurrence in rice-fields, and in waste from starch factories, &c. The occurrence of one of these forms in the sulphuretum may be accidental. It was thought advisable, however, to mention and characterize the Athiorhodaceae, as considerable confusion still reigns as to their true nature (Skene, Waksman, and Joffe, &c.).

VII. *Other members of the S cycle.* A *Diplococcus* was isolated from a *Lamprocystis sulphuretum* (mud lake) near Portola, California, on Beyerinck's thiosulphate medium.[1] It causes an abundant deposit of sulphur on the surface of the jar. The Owen's Lake cultures also were covered with sulphur, which was swarming with small *Spirilla* (exothiobacteria ?).

Sulphate reduction was observed in a filtered brine solution with the formation of black mud. In the mass cultures, most of the black mud formation may be due to either protein or sulphur decomposition. In the case investigated, however, the brine contained only a small amount of organic matter, insufficient to account for the large amount of black mud formed. The mud is formed at the surface of the jar, where a film of iron hydroxide is present (due to the insolubility of Fe^{++} ion at pH 7). The FeS formed proceeded to sink to the bottom, and was partly oxidized on its way. This went on until a heavy black sediment was formed on the clear brine solution. Only at this point the sulphur bacteria came into play (fourteen days after inoculation).

The following schematical representation can be given of the conditions that dominate the organic balance in the sulphuretum :

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[1] In a flask, incubated at 25° C. After five days a skin of S begins to form.

AUTOTROPHIC

Chemosynthetic
{
Beggiatoa, Thiothrix
Exothiobacteria
Thiorhodaceae ?

Photosynthetic
{
Blue-green algae
Green algae
Euglenoids
Green bacteria ?

HETEROTROPHIC
Bacteria causing putrefaction
Sulphate reducers
Athiorhodaceae
Spirochaete ?
Ciliates
Amoebae
Nematodes.

It may be that the cycle would run somewhat like this:
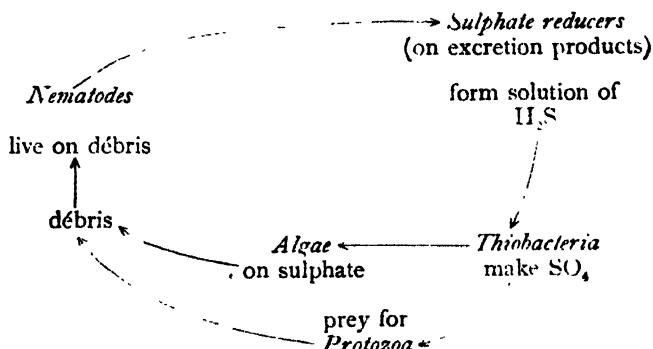
The cycle must be much more complicated than this. However, a fuller treatment of this phase lies outside the scope of this paper. The chief reason for the studies outlined in this section was *to emphasize the specific nature of the sulphuretum.*

C. THE INTERNAL MILIEU.   THE MECHANISM OF OXIDATION.

I. *The Equilibrium of H_2S in water.*

It has been found that H_2S is soluble in distilled water to a concentration of about 0·102 molar at 25° C.[1] in a H_2S pressure of 1 atm. This solubility will change, however. when there are metal ions in solution.

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[1] Winkler, Z. f. Physik. Chem., lv. 350 (1900); Kendall and Andrews, J. Am. Chem. Soc., xliii. 1545 (1921).

In order to determine the conditions existing there, we have to consider the equilibrium according to ionic theory and mass law.

In the first place, the H_2S will be dissociated in hydrosulphide ion and hydrogen ion, according to

$$K_1 (H_2S) = (H^+) (HS^-) \dots\dots\dots\dots (1)$$

in which K_1 is about 0.91×10^{-7} at $18°$ C.[1]

The second dissociation constant K_2 in

$$K_2 (HS^-) = (H^+) (S^{--}) \dots\dots\dots\dots (2)$$

is so very small that even in moderately alkaline solution a normal sulphide is largely hydrolysed. K_2 must be of the order of $K_2 = 1 \times 10^{-15}$ at laboratory temperatures.[2]

The sum of the positive charges (total metal ion concentration + hydrogen-ion concentration) must equalize the sum of the negative charges (sulphide, hydrosulphide, and hydroxyl ion).

$$(B^+) + (H^+) = (OH^-) + (HS^-) + 2 (S^{--}) \dots\dots\dots\dots (3)$$

We also have the relation between hydroxyl and hydrogen ions.

$$(H^+) (OH^-) = K_w \dots\dots\dots\dots\dots (4)$$

From those four equations we can solve for H_2S, and expressing this in H^+ and B^+ we get:

$$(H_2S) = \frac{\{(B^+) + (H^+)\} (H^+)^2 - K_w (H^+)}{K_1 (H^+) + 2 K_1 K_2} \dots\dots\dots (5).$$
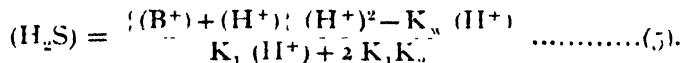
Equation (5) enables us furthermore to calculate the pH of a 0.102 M solution of H_2S in distilled water, $B^+ = O$. Equation (5) becomes

$$\frac{(H^+)^3 - K_w (H^+)}{K_1 (H^+) + 2 K_1 K_2} = 0.102, \text{ or}$$

$$(H^+)^3 - 9.8 \times 10^{-9} (H^+) - 1.8 \times 10^{-23} = 0.$$

Solving by the Cardanus method we find only one real root for the equation: $H^+ = 7.8 \times 10^{-5}$. The amount of H_2S in waters containing metal ions will be calculated on the assumption that the metal ions are present in very low concentration: $B^+ = 10^{-5}$. Solving from (1), (2), and (5) we get: $B^+ = 10^{-5}$.

H+	H₂S	HS⁻	S⁻⁻
10^{-5}	2.2×10^{-3}	2×10^{-5}	2×10^{-15}
10^{-6}	1.2×10^{-4}	1.1×10^{-5}	1.1×10^{-14}
10^{-7}	1.1×10^{-5}	1×10^{-5}	1×10^{-13}
10^{-8}	1×10^{-6}	9.1×10^{-6}	9.1×10^{-13}
10^{-9}	2.2×10^{-8}	2×10^{-6}	2×10^{-12}

[1] Auerbach, Z. f. Physik. Chem., xlix. 217 (1904).
[2] Knox, Trans. Faraday Soc., iv. 29 (1908).

In order to present this graphically we will plot the concentrations as C_H, C_{HS^-}, C_{H_2S}, and $C_{S^{--}}$ —

CH	CH$_2$S		CS^{--}
	−2·66	−4·79	−14·70
−6	−3·92	−1·96	−13·96
−7	−4·96	−5·00	−13·00
−8	−6·00	−5·04	−12·04
	−7·66		−11·70

Text-fig. 2 shows this graphically. The ordinates show HS⁻, H₂S, and S⁻⁻, while the abscissae indicate (H⁺). It can be seen that in a
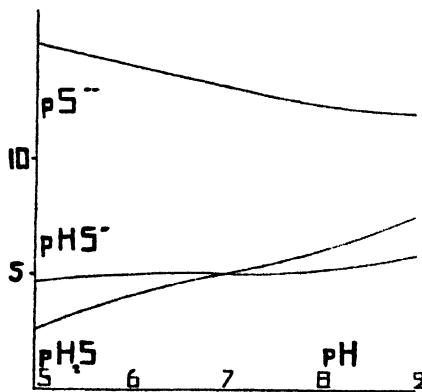
TEXT-FIG. 2.

sulphuretum (pH 7·6–8·6) the HS⁻ concentration is 50–100 times as high as the H₂S concentration. The sulphide concentration is very low throughout (one ten-millionth of the hydrosulphide in the sulphuretum).

The equilibrium conditions treated above dominate all solutions of H₂S, whether containing base or not. The contradictory results in the literature on the solubility of calcium sulphide can be accounted for by the lack of accurate knowledge of the dissociation constants[1]

As the atmospheric pressure of the H₂S is usually zero, the solution will lose its H₂S rapidly by diffusion, especially on aeration. The equilibrium will be destroyed until the conditions of temperature, aeration, and H₂S supply are constant again.

It is certain that no more than two parts of CaS per 1,000 of water can be dissolved in any form whatsoever. $B^+ = 2·5 \times 10^{-2}$. Skene (loc. cit., p. 13), in studying endothiobacteria, found 0·5 per cent. CaS not suitable as a medium. This is about 50 per cent. CaS insoluble, and must have kept the water about saturated with H₂S, a condition toxic for the bacteria. However, Mlle Hannevart, in a remarkable study of the endo-

[1] Kolb, Ann. Chim. (4), vii. 126; Béchamp, ibid., xvi. 222.

thiobacterium *Achromatium oxaliferum*, Schew. (= *Hillhousia mirabilis*, West and Griffiths, = *Modderula Hartwigi*, Frenz), has been able to cultivate this form in a solution of CaS.

The author has found very small doses of CaS (0·01 per cent.) beneficial to the growth of *Lamprocystis*. This is self-evident from the preceding pages. The CaS, being a salt of a weak acid and a strong base, will be mostly hydrolysed and give rise to SH^- and H_2S. The amount of H_2S in equilibrium with the base at different concentrations is, for a pH = 8 (from equation (5)):

		H_2S.	p.p. 1000.
$B^+ = 10^0$	1 (Brine)	$1\cdot1 \times 10^{-1}$	3·8
$B^+ = 10^{-1}$	(Owen's Lake)	$1\cdot1 \times 10^{-2}$	$3\cdot8 \times 10^{-1}$
$B^+ = 10^{-2}$		$1\cdot1 \times 10^{-3}$	$3\cdot8 \times 10^{-2}$
$B^+ = 10^{-3}$	(Searsville)	$1\cdot1 \times 10^{-4}$	$3\cdot8 \times 10^{-3}$
$B^+ = 10^{-4}$		$1\cdot1 \times 10^{-5}$	$3\cdot8 \times 10^{-4}$
$B^+ = 10^{-5}$		1×10^{-6}	$3\cdot4 \times 10^{-5}$

Any amount of H_2S in excess of this is not in equilibrium with the metal ion. This fact may account for the toxicity of high H_2S and optimum concentration for this gas in *Beggiatoa*.[1]

Distilled water in contact with one atmosphere H_2S contains about $(H_2S) = 0\cdot102$ M. If we know the partial pressure of the H_2S, in which the most luxuriant endothio vegetation occurs, we shall be able, by Henry's law,[2] to calculate the molarity of H_2S in distilled water.

Keil (loc. cit., p. 343) found the H_2S optimum for *Beggiatoa* about 0·8 mm. of mercury. This is 1/9500, or about $1\cdot1 \times 10^{-4}$ atmospheres. Distilled water, in contact with such an atmosphere, will contain an approximately 1×10^{-5} molar solution of $H_2S + HS^-$.

At a higher pH, in a hard water, less H_2S will exist in solution (see below). It is possible to calculate, from total metal concentration and pH, the amount of H_2S in equilibrium with this base.

The salt concentrations in Keil's nutrient solution (imitation of Winogradsky's Langenbrücker water) are:

$Ca(HCO_3)$	0·34 %	concentration Ca^{++}	0·112 %
$CaSO_4$	0·31 %	,,	,,	0·091 %
$Mg(HCO_3)$	0·27 %	,,	Mg^{++}	0·053 %
$MgSO_4$	0·51 %	,,	,,	0·170 %
Na_2SO_4	0·21 %	,,	Na^+	0·060 %

(Ca^{++}) 0·028 M. + 0·023 M. = 0·052 molar
(Mg^{++}) 0·022 M. + 0·071 M. = 0·093 molar
(Na^+) 0·026 M.   = 0·026 molar

0·170 molar.

[1] F. Keil, Cohn's Beitr. zur Biol. d. Pflanzen, vii. 312 (1912).
[2] Lewis and Randal, p. 543.

The equation

$$\frac{\{(B^+) + (H^+)\} (H^+)^2 - K (H^+)}{K_1 (H^+) + 2 K_1 K_2} = (H_2S)$$

for high base concentration becomes :

$$\frac{(B^+) (H^+)}{K_1} = (H_2S) \quad \text{or} \quad (B^+) = \frac{K_1 (H_2S)}{(H^+)}$$

$$(B^+) = 9 \cdot 1 \ 10^{-8} \frac{(H_2S)}{(H^+)},$$

supposing that all the base is bound to (SH⁻). Only a small amount of the 0·17 molar total base will be bound to H_2S. The amount in actual relation between the base and different acids will be determined by the proportion of the dissociation constants of these acids,[1] and also by their concentration. It would be a complicated task to unravel this for Keil's culture solution.

It is evident, therefore, that only a small fraction of the B^+ would suffice to keep the H_2S in solution.

Keil's H_2S or 10^{-5} would give, at a H^+ of 10^{-8} :

$$B^+ \text{ or } SH^- = 9 \cdot 1 \times 10^{-8} \frac{10^{-5}}{10^{-8}}$$

$$B^+ = 9 \cdot 1 \times 10^{-5}.$$

As the culture solution of Keil must have a pH around 8, we see that only about 10^{-4}, one ten-thousandth of the available metal, takes part in the fixing of the H_2S. The results check well with those derived for a hypothetical case above, and there is evidence, therefore, to assume that *Beggiatoa* has its H_2S (or rather SH^-) optimum when all these substances are in true solution. If there is more, the poisonous H_2S in excess of the SH^- will make its presence known. It is quite possible, therefore, that H_2S is poisonous to sulphur bacteria as much as to other organisms, and that Ostwald's statement that H_2S is not poisonous for the S bacteria ' which generate it ' (loc. cit., p. 312) is erroneous in two ways.

II. *Source of energy of the thiobacteria.*

In any process occurring at constant temperature and pressure, the maximum of work which can be obtained from a given process and applied to useful purposes is called ΔF, in which F is known as the *free energy*.

This free energy change bears the same relation to the total external work done by the system as a heat of reaction is related to a total energy content.

Total external work done by the system ΔA is equal to $\Delta E - T\Delta S$;

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[1] Nernst, Theoretische Chemie, 5th edition, p. 600, 1921.

change in total energy content, minus heat absorbed, ΔS being the entropy change, ΔS heat absorbed divided by absolute temperature.

In representing the relation between members of a cycle, such as the carbon, nitrogen, or sulphur cycles, the change in free energy (or maximum of work which can be obtained from a given process) has to be used to characterize a change in an external or internal metabolite. Obviously this change in free energy is a better measure than a 'heat of reaction'; the latter including such diffuse energy as will increase the entropy (or reduce the heat quantity) of the system.[1]

Let us try to apply this principle in order to find out whether H_2S can be the source of energy for the thiobacteria. After Winogradsky's classical work nobody has contested this idea as far as the author is aware.[2]

The fact that, in hard waters, the concentration of the SH^- ion is so much higher than the H_2S concentration justified the suspicion that H_2S is after all not the source of the S droplets in or outside the cell. In the modern text-books H_2S is described as a 'source of energy'. The recent monographs on the subject (cited above) are equally unanimous on this point.

As a matter of fact, the reaction $H_2S = H_2 + S$ is endothermic, requiring 4,800 calories ΔH, or heat of reaction. It is the simultaneous oxidation of the hydrogen that makes the net reaction exothermic. On the other hand, the reaction $H_2 + S = H_2S$ is, of course, exothermic, and is performed by various living cells, such as yeast, as treated elsewhere in this paper. Heat of reaction, as mentioned above, is no absolute index of the energy change, however. It is the decrease in free energy (ΔF) that really matters, and reactions in which ΔF is negative (or in which the entropy increases) will be those that will be met with in most of the simple vital oxidations and reductions. The *a priori* expectation of the rôle of the SH^- ion becomes clear when we study the energy changes in sulphur compounds as given by Lewis and Randall (loc. cit., p. 531):

$$HS^- = S^{--} + H^+, \qquad \Delta F^\circ \; 298 = \qquad 20470 \text{ cal. } (1)$$
$$H_2S(Aq) = HS^- + H^+, \qquad ,, \quad ,, = \qquad 9470 \text{ cal. } (2)$$
$$H_2 + S(rh) = H_2S \text{ aq}, \qquad ,, \quad ,, = \quad -6490 \text{ cal. } (3)$$
$$S(rh) + 2O_2 + 2^{--} = SO_4^{---} \;,, \quad ,, = \;-176500 \text{ cal. } (4)$$
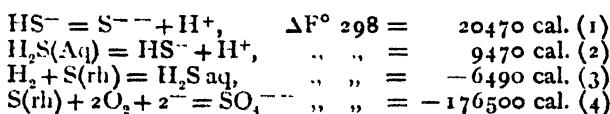
From this set of data the free energy levels of the different compounds can be calculated by plain addition. Text-fig. 3 shows the different energy levels. (The free energy change from liquid sulphur to rhombic sulphur is so small that it does not appear in a figure on this scale.)

---

[1] See for a discussion of this principle: (1) G. N. Lewis and U. Randall, Thermodynamics and the Free Energy of Chemical Substances, New York, 1923, *passim*, and especially chapter xiv; (2) M. Planck, Treatise on Thermodynamics, English edition, London, 1917, p. 110; (3) P. H. Kohnstamm, Warmteleer, Amsterdam, 1915, p. 122.

[2] See, however, G. Nadson, Bull. Jard. Bot., Pétersbourg, xii. 83 (1912), who claims H_2S not to be a 'condition *sine qua non*' for the bacteria.

It will be seen at a glance that the aqueous solution of H_2S has a lower free energy level than the sulphur, and that hydrogen·sulphide is therefore unfit to serve as a simple source of energy according to the second law of thermodynamics.

The sulphide ion has the highest free energy level. Its extremely low concentration in natural waters, however, lessens its importance considerably. It seems that there is sufficient evidence to warrant the conclusion that the *hydrosulphide ion and not the free hydrogen sulphide is used by the sulphur bacteria as a source of energy.*

For those who consider the Thiorhodaceae to be both photosynthetic and chemosynthetic, there is one other possibility. It is possible that these forms liberate enough energy by combustion of photosynthate to raise the energy level of H_2S to that of free sulphur. They would be able to use this S as photosynthate. This reasoning would break down, however, after consideration of the colourless forms, such as *Beggiatoa*, which are decidedly not capable of photosynthesis. It is true that Maxwell's demon, the being who is able to diminish the entropy of a system, has been materialized by Helmholtz in the form of a micro-organism. Lewis and Randall (loc. cit., p. 121) state that in this case 'it is conceivable that systems might be found in which these micro-organisms would produce chemical reactions where the entropy of the whole system, including the substances of the organisms themselves, would diminish. Such systems have not as yet been discovered, but it would be dogmatic to assert that they do not exist.'

In the case of the sulphur bacteria, according to the writer, it would be dogmatic to assert that they do exist. Opponents of the view as outlined in this last section have, however, recourse to this (vitalistic) possibility.

III. *Origin of the soluble liquid sulphur.*

In nature the soluble liquid sulphur appears by sublimation of rhombic sulphur, by oxidation of polysulphide, or by oxidation of an aqueous solution of hydrogen sulphide. In the latter case the reaction takes place in the air, and is accelerated by nitrate, bichromate, or permanganate ions, all reducible ions. As far as the author is aware, the influence of the oxygen tension on this process has never been studied. However, O_2-free H_2S in boiled distilled water will reduce methylene blue. This fact will be used to account for the mechanism of oxidation.

Diagram (left margin):

'99950 — S⁻

'79480 — HS⁻
'76500 — S
'70000 — H₂S aq

ΔF°_{298}

0 — SO₄⁻⁻

TEXT-FIG 3.

Winogradsky (loc. cit.) has proved that the sulphur in the bacteria is actually the Sλ or soluble liquid sulphur. The other existing form of liquid sulphur, Sμ, is not soluble in carbon bisulphide.[1] The 'liquidity' of the sulphur may be shown on heating the bacteria carefully; large coalesced droplets fill the cell. Their shape is often irregular, deformed by outside pressure. In the photograph of *Beggiatoa* (Pl. XVI, Fig. 2) this can be readily seen.

From the inorganic analoga we are led to believe that a reducible substance, such as methylene blue, potassium bichromate, or nitrate, present in an aqueous solution of H_2S (and its dissociation products) will 'dehydrogenate' the HS^- to liquid sulphur. In the case of the endothiobacteria this reducible substance will be present inside the cell, in case of the ectothiobacteria it will be secreted by the cell.

The oxygen relations of the sulphuretum give another clue for the explanation of the oxidation. Water containing H_2S is poor in oxygen, especially hard water; in which the oxygen will be used in oxidizing the sulphide. There is no free oxygen in the normal sulphuretum, and although Thiorhodaceae are often living in water containing oxygen (*Lamprocystis*, observed by the author), it is clear that they cannot derive the oxygen from the surrounding water. Nadson (loc. cit.), observing the oxidation of black mud around Chlorophyceae in the light, fails to see such a bleaching around S bacteria. Molisch fails to observe any attraction for bacteria of the 'thermo' group in the light. Buder (loc. cit., p. 580), after a careful survey of the literature, comes to the opposite conclusion. According to him, traces of oxygen *may* enter into the surrounding medium. The ecological rôle of the green bacteria in the sulphuretum seems to be problematical. Winogradsky looks upon them as possible sources of oxygen for the S bacteria, but their numbers would hardly be sufficient to create an appreciable oxygen tension. Moreover, they are absent in the marine sulphureta. This was particularly striking in the brine from Redwood City.

For the 'H_2S' oxidation, at least, Buder allows for an intracellular oxidation. The S oxidation will be treated below.

It seems that, in the absence of free oxygen, one is driven to accept some sort of oxidative process as proposed by Wieland[2] and Hopkins.[3] Wieland looks upon oxidation as dehydrogenation. The oxidation of carbon monoxide, for instance, has been shown to take place according to the following scheme (the intermediate products have been isolated):

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[1] Hannevart (loc. cit.) seems to have found CS_2 insoluble sulphur in *Achromatium oxaliferum*.

[2] H. Wieland, Chem. Ber., xlvii. 2085, 1914. See also H. D. Dakin, Physiol. Reviews, i. 394, 1921.

[3] F. G. Hopkins, Biochem. Journ., xv, 1921.

I. $CO + H_2O = HCOOH$ formic acid.
II. $HCOOH = H_2 + CO_2$ dehydrogenation.
III. $H_2 + O_2 = H_2O_2$ hydrogen acceptance.
IV. $H_2O_2 = H_2O + 1/2 O_2$.

$CO + 1/2 O_2 = CO_2$ net result.

In Stage III, hydrogen acceptance, methylene blue, or other reducible substances may take the place of the oxygen.

Hopkins has isolated a substance which he calls glutathione, a compound containing cystin (cystein) and glucosamin. This substance is extremely abundant in yeast, and has the properties of a hydrogen acceptor.

$$
\begin{array}{ccc}
CH_2-S-S-CH_2 & & \\
| \qquad\qquad | & & CH_2SH \\
CHNH_2 \quad CHNH_2 & & | \\
| \qquad\qquad | & +2H \rightleftharpoons & CHNH_2 \\
C=O \qquad C=O & & | \\
\diagdown \qquad\quad \diagdown & & C=O \\
R \qquad\quad R & & \diagdown \\
& & R
\end{array}
$$
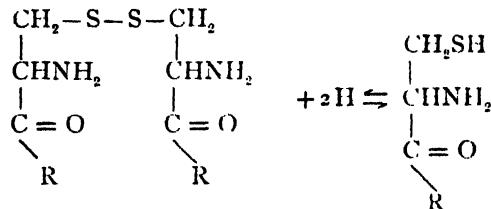
At a pH of 6·8 or lower the cystin, becoming cystein through hydrogen acceptance, *will not* transfer this hydrogen to methylene blue and reduce it to the leuko base. At a pH of 7·4 or higher the cystein, becoming cystin through hydrogen acceptance, *will* transfer this hydrogen to methylene blue and reduce it to the leuko base.

The energy liberated in the oxydation of the SH^- group will be used to reduce CO_2. This reduction takes place in an alkaline medium ; according to Hopkins, transference of the hydrogen from the reduced glutathione is possible.

The extreme indifference to salt concentration of the thiobacteria may be due to total permeability for certain ions. In that case there would be no difficulty in explaining the penetration of the HS^- ion into the cell. This series of reactions (which is given entirely in a tentative way) would look as follows :

$$
\begin{array}{ccc}
CH_2-S-S-CH_2 & & \\
| \qquad\qquad | & & CH_2-S-H \\
CH.NH_2 \quad CH.NH_2 & & | \\
| \qquad\qquad | & +2HS^- = 2 & CH.NH_2 \quad +S+2E \\
C=O \qquad C=O & & | \qquad\qquad \downarrow \\
\diagdown \qquad\quad \diagdown & & C=O \quad \text{negative charge} \\
R \qquad\quad R & & \diagdown \\
& & R
\end{array}
$$

$$
\begin{array}{lll}
\mathrm{CH_2-S-H} & \mathrm{CH_2-S-S-CH_2} & \text{formaldehyde} \\
2\,\mathrm{CH.NH_2+H_2CO_3} = & \mathrm{CH.NH_2 \quad CH.NH_2} & \\
\mathrm{C=O} & \mathrm{C=O \qquad C=O} & +\mathrm{HC=O} \\
\diagdown & \diagdown \qquad\quad \diagdown & \mid \\
\mathrm{R} & \mathrm{R} \qquad\quad \mathrm{R} & \mathrm{H}
\end{array}
$$

$$+\,\mathrm{H_2O_2}$$

II peroxide

$$\mathrm{H_2O_2 + 2E = H_2O + 1/2\,O_2 + 2E}$$

negative charge   negative charge
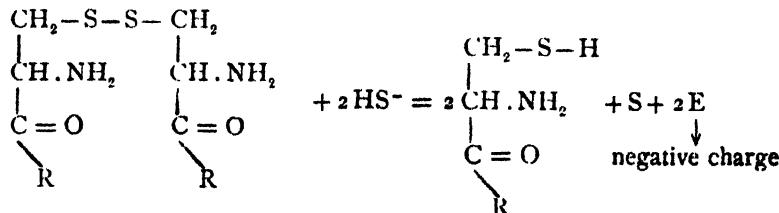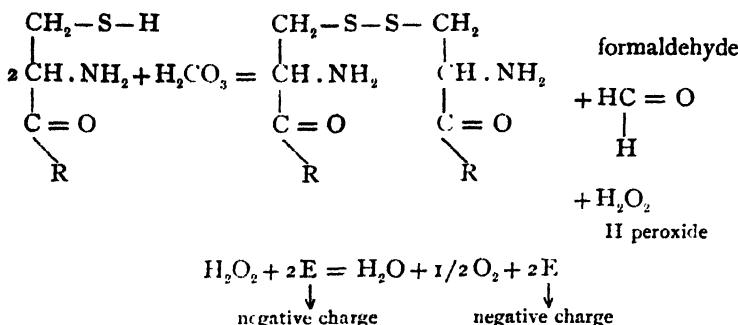
The O_2 developed can be used inside the cell to perform work, especially to oxidize the S to SO_4^{--}. It is, however, evident that in the case of *Beggiatoa*, the external oxygen plays a rôle in this process (Winogradsky, Jegunov, Keil). It is evident that S oxidation and S formation are two simultaneous processes. In the absence of the latter, however, the former will make its effect known more clearly. If the glutathione, or a similar substance, acted as a hydrogen acceptor, the hydrogen would have to be shifted to another compound soon, as the following considerations will show.

Suppose a *Spirillum* of 2μ wide, 30μ long, containing 4 globular S droplets with a diameter of 2μ. The total volume of the *Spirillum* will be approximately $100\mu^3$, the total volume of its droplets $4 \times \pi 4/3\,r^3 = 16\mu^3$.

Volume *Spirillum*	$100\mu^3$		
Volume S droplets	$16\mu^3$	weight approx.	$2 \cdot 9 \times 10^{-11}$ grm.
Volume living matter	$84\mu^3$		
Water content $\pm 80\,\%$	$67\mu^3$	,, ,,	$6 \cdot 7 \times 10^{-11}$,,
Protoplasm $\pm$ salts	$17\mu^3$	,, ,,	$2 \cdot 6 \times 10^{-11}$,,

Total weight *Spirillum* $12 \cdot 2 \times 10^{-11}$ grm.

The weight of the sulphur is equal to the weight of the dry matter. Moreover, for 33 grm. of SH^-, 32 grm. are sulphur, and only 1 grm. H enters the non-reduced glutathione. As the molecular weight of the reduced glutathione molecule must be at least several hundreds, it can be seen that there must be many more S molecules in the body of the bacterium than molecules of the cystein derivatives. Accordingly the glutathione has to play the rôle of a catalyst or co-enzyme.[1] The above theory of SH^- oxidation and CO_2 assimilation in the Thiorhodaceae is in harmony with existing knowledge of physiological oxidations, as well as with the alkaline conditions of the sulphuretum. It is also adapted to account for the possible utilization of a large amount of SH^- ion by a small

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[1] On related matters see H. D. Dakin, Physiol. Reviews, i. 3, p. 394, 1921.

amount of living matter. Yeast, in contact with free sulphur, liberates H_2S. De Ray Pailhade claims this action to be due to an enzyme, thionase. However, the change of S to H_2S is easily performed by a great variety of cells and tissues. A reaction such as this, performed with extreme ease (as a matter of fact, going on spontaneously), goes together with a decrease in free energy (see Text-fig. 3). The opposite reaction, also in aqueous medium, as taking place in the thiobacteria has been assumed to be able to perform work as well (Winogradsky's theory). If H_2S were used by the sulphur bacteria one would be driven to accept, by virtue of our experience with yeast, the existence of a *perpetuum mobile.*[1]

The impossibility of a reversible reaction on which the free energy change would be a measurable quantity, as exemplified by the H_2S oxidation and the sulphur reduction, gives additional support to the theory of HS^- dehydrogenation. We leave, therefore, according to the Wieland-Hopkins theory, the sulphur bacterium with : (1) free sulphur derived from this dehydrogenation ; (2) oxygen derived intramolecularly from bicarbonate or peroxide ; (3) two negative charges. The fate of this system will be considered in the paragraph on sulphur oxidation. In the beginning of this work an abundant mass culture of *Lamprocystis* was obtained, which, on grinding with sand and extracting with water, yielded, after precipitation, a greyish powder that when in solution oxidized H_2S quite actively in a eudiometer tube (Pl. XVI, Fig. 11). Nor did it lose this property after dialysis for twenty-four hours in distilled water. The oxidizing power was affected, but not destroyed, at $80°$ C. Boiling reduced the oxidizing activity to that of distilled water.

These scanty results do not warrant a conclusion as to the nature of this press juice, which was studied before the proper equilibrium relation of H_2S in water was understood. The presence of an ecto-enzyme ' thionase ' is therefore not proved. Still there seems to be experimental evidence of the presence of a hydrogen acceptor (in Wieland's sense) in the sulphur bacteria.

IV. *Relation between soluble, liquid sulphur, and rhombic sulphur.*

Sulphur can exist in a great many allotropic modifications. The stable modification, at ordinary temperature, is rhombic sulphur. According to Ostwald,[2] this rhombic sulphur is the only form existing in nature. We have seen, however, that the soluble liquid exists inside the cells of the endothiobacteria.

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[1] See on the *perpetuum mobile*, Kohnstamm, loc. cit., p. 78. The author has tried, by the iodine-thiosulphate method, to titrate the amount of H_2S liberated by yeast from S. The values obtained were very much too high, possibly due to the development of another ' dehydrogenizible ' compound.

[2] W. Ostwald, Grundlinien der anorganischen Chemie, 3rd edition, 1913, Dresden, p. 297.

The pressure-temperature diagram of sulphur phases is given in Text-fig. 4. At ordinary temperatures, liquid and monoclinic sulphur are metastable modifications, and will pass readily in the rhombic modification under ordinary conditions. The stable phase has the lowest vapour pressure.[1] Sulphur droplets in a test-tube, prepared by sublimation, will begin to distil over towards the larger droplets, the latter having the lowest vapour pressure. After a few hours most of these larger droplets will have begun to crystallize out (Oswald, loc. cit., p. 290). We would expect, from the pressure-temperature diagram, that the liquid sulphur
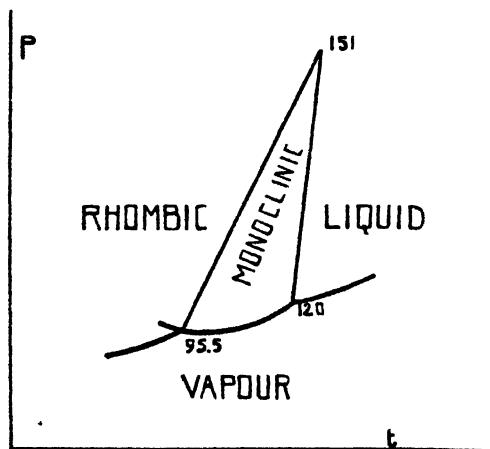
Tᴇxт-ᴛɪɢ. 4.

(unstable below 120° C.) would first become monoclinic (unstable below 95°), and only after a sojourn in this phase begin to become rhombic. However. in the experiment described above, the monoclinic phase is omitted from the process, and the droplets crystallize out in the rhombic system.

Liquid sulphur can be kept in semi-permanent slides of dead sulphur bacteria in a concentrated solution of cane sugar, or in an agar solution. In other conditions they will change to the rhombic form. In Winogradsky's method the bacteria are killed by heating them on a slide to about 80° C. A cover-slip with the bacteria was placed for two minutes on a hot plate. It can be seen immediately that the droplets join (Pl. XVI, Fig. 6 for *Spirillum*, Fig. 2 for *Beggiatoa*, Fig. 13 for *Chromatium*). Next day the sulphur is crystallized out in the rhombic system (see Pl. XVI, Figs. 3, 9). The transition liquid to rhombic sulphur means a free energy drop of ninety-four calories.

In certain types the Sλ could not be changed to the rhombic form

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[1] For the reason of this see Lewis and Randall, loc. cit., chapter xvii, ' The Fugacity '.

at all after killing the bacteria by heat. Freshwater *Chromatium*, for instance, kept the droplets for a long time, while *Beggiatoa, Lamprocystis*, and *Thiospirillum* showed the typical octahedra after twelve to twenty-four hours.

The Chromatia from the brine, however, yielded crystals which, in habit, looked strikingly like monoclinic sulphur (Pl. XVI, Fig. 14).

Examination under crossed nicols, however, showed that the sulphur had actually passed to the rhombic state, and that the crystals were, therefore, pseudomorphoses.   Dr. Edgar S. Wherry, of the Bureau of Chemistry, Washington, D.C., has had the kindness to comment upon this fact in a written communication.   According to him, the needle-shaped crystals may simply be rhombic crystals in which certain planes are suppressed by impurities.   NaCl, in alkaline medium, for instance, will crystallize out in octahedra instead of cubes.   The hydroxyl seems to have the tendency to deposit on the octahedral faces of the first crystal nucleus.   If the needle-shaped crystals had been originally monoclinic, but rearranged themselves into the rhombic system, we would have a case analogous to that of calcium carbonate, crystallizing in the trigonal system below 70° C. (calcite), and in the rhombic system above 70° C. (aragonite).   In sea-water, however, rhombic aragonite crystallizes out at ordinary temperatures.   This is probably effected by the sulphate in the sea-water.

Pseudomorphic rhombic sulphur is found in nature.[1]   It may be that in the free energy drop of ninety-four calories between Sλ and S rhombic, the intermediate stage (S monoclinic) is met with in certain cases.

V. *The oxidation of the sulphur.*

The oxidation of the sulphur has been studied by Winogradsky for endothiobacteria, by Waksman for *Thiobacillus thio-oxidans*.   In the latter was found an enormous increase in acidity, up to a pH of 0·6— the highest acidity known to be tolerated by any living organism.   The conditions existing in the endothio forms is, of course, quite different.   The large buffer value of the hard-water medium (see above) will completely neutralize the acidity of the sulphuric acid formed.

In both the endo- and ecto-forms, however, the sulphur will be oxidized according to

$$H_2 + S\lambda + 2\,O_2 = H_2SO_4\,(Aq)';\quad \Delta F^\circ_{298} = 176500$$

or

$$S\,(\lambda) + 2\,O_2 + 2\,E = SO_4{}^-;\quad \Delta F^\circ_{298} = -176500,[2]$$

$\uparrow$
negative charge
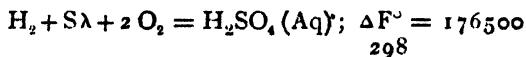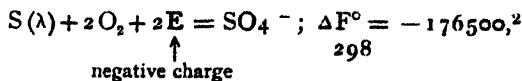
[1] Private communication of Dr. A. F. Rogers.
[2] Lewis and Randall, loc. cit., p. 555.

now recalling the proposed mechanism of hydrosulphide dehydrogenation :

$$
\begin{array}{ccc}
CH_2-S-S-CH_2 & & CH_2SH \\
| & | & | \\
CH.NH_2 & CH.NH_2 +2HS^- = 2\,CH.NH_2 & +2S\lambda+2\,\dot{E}\,\dots\dots\dots\dots(A) \\
| & | & | \\
C=O & C=O & C=O \\
\diagdown & \diagdown & \diagdown \\
R & R & R
\end{array}
$$

$$
\begin{array}{cccc}
CH_2SH & CH_2-S-S-CH_2 & & \\
| & | & | & \diagup\!\!O+H_2O_2\dots\dots(B) \\
CH.NH_2+H_2CO_2 = CH.NH_2 & CH.NH_2 & +HC & \\
| & | & | & | \\
C=O & C=O & C=O & H \\
\diagdown & \diagdown & \diagdown & \\
R & R & R &
\end{array}
$$

$$H_2O_2 = H_2O+1/2\,O_2 \dots\dots\dots\dots\dots(C)$$

$$
(A)+(B)+(C) = 2HS^-+H_2CO_3 = 2S+H\overset{\overset{\textstyle O}{\|}}{C}+H_2O+1/2\,O_2+2\,\dot{E}\ \dots\dots(D)
$$
$$\underset{H}{|}$$
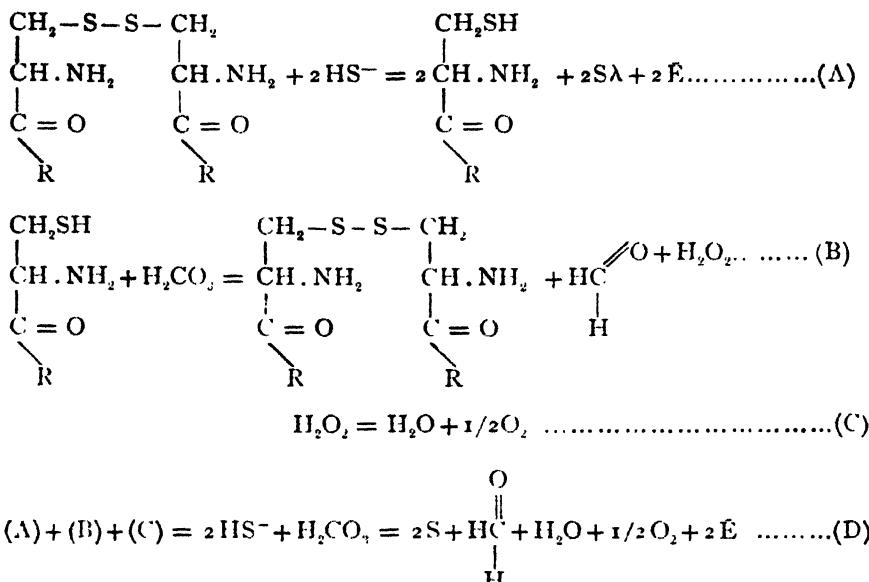
The formaldehyde will be used for building carbohydrates. There seems to be not enough oxygen to oxidize the sulphur to sulphate according to the formula used above. The sulphur will remain in the cell until the HS⁻ in the solution is exhausted. The water will become saturated with oxygen, and the amount of this gas will suffice to oxidize the sulphur to sulphate.

According to Winogradsky (who discovered this phenomenon · the high Ca-requirement of the sulphuretum is due to the necessity for neutralization of the H_2SO_4 formed. However, according to Lewis and Randall's formula, it is more probable that S will be oxidized to primarily the sulphate ion. The interior of the protoplasm of the endothio forms remains alkaline throughout, as is proved by the presence of $CaCO_3$ granules in certain cases,[1] and the high catalase content demonstrated by the author. The latter fact is of importance in view of the possibility of a 'Wieland' mechanism, in which the presence of a catalase is required, as well as to show the alkalinity of the cellular contents. Catalase is inactive in acid solutions.

The 'starvation phenomenon' of S oxidation, as described by Winogradsky, could be easily observed in *Beggiatoa, Thiothrix*, and in

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[1] E. Bersa, Sitzb. d. Akad. Wiss. Wien, cxxix. I, 1920; G. S. West and B. Griffith, this Journal, xxvii. 83, 1913.

Thiospirillum. In *Chromatium*, however, all attempts failed. In order to facilitate the S oxidation, the *Chromatium* was studied in artificial acid medium. In one set of experiments the acidity was increased by bubbling CO_2 through the culture fluid till the water was decidedly acid (pH = 6).
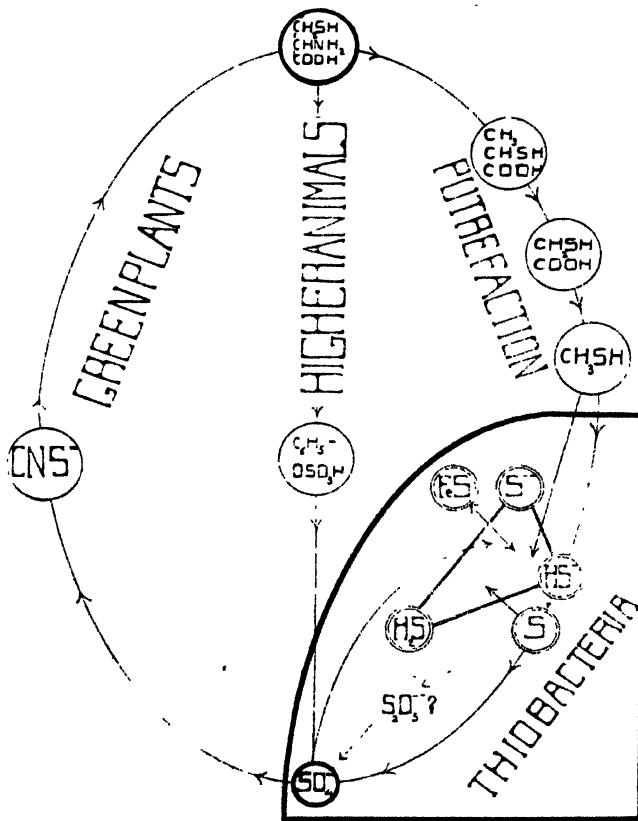
TEXT-FIG. 5.

After a sojourn of forty-eight hours in this acid liquid, the Chromatia were still living and contained copious S droplets.

To another culture a phosphate buffer was added, bringing the pH down to 6, after considerable calcium phosphate precipitation. After twelve hours the Chromatia were all dead; the sulphur droplets, however, still intact.

The oxidation of the sulphur in *Chromatium* could, therefore, not be accelerated by increased H-ion concentration. As there is evidence that the endothio forms are permeable to salt ions of various kinds, we have reason to assume that $CaSO_4$. $2H_2O$ will be the oxidation product. Here

we have an example of an important link in the sulphur cycle, the carbonate-sulphate relation.[1]

D. CONCLUSIONS.

The chief result of this paper, the inadequacy of hydrogen sulphide as a source of energy, only amounts to the change of one word in Winogradsky's results. The general meaning of his beautiful discoveries remains unaltered. In the schematical representation of the sulphur cycle on p. 648 (Text-fig. 5) the 'inorganic' part of the cycle constitutes the realm of the thiobacteria.

It can be seen from this chart that cystein (cystin) and sulphate constitute the opposite poles of the cycle. Many intermediate stages (chondroitin, myrosin, thiosulphate, taurin, &c.) are omitted so as not to complicate the scheme. In this cycle we find the black mud in the rôle of mineral reserve; an almost inexhaustible storehouse for HS^- ion in alkaline solution.

E. SUMMARY.

1. The term *endothiobacteria* was devised for the forms which deposit soluble liquid sulphur inside the cell. These forms occur in a distinct community, called *sulphuretum*.

2. The sulphuretum is apparently limited to alkaline water. It is independent of salinity, this factor varying from 0·05 per cent. to 7·5 per cent.

3. The forms depend on some solution product of H_2S. They are oligo-aerophilous, occurring together with anaerobic and polysaprobic forms.

4. Nine genera of the endothiobacteria were found in the vicinity of Stanford University.

5. The oxidation of hydrogen sulphide cannot be the source of energy for the sulphur bacteria.

6. The dehydrogenation of the hydrosulphide ion is the probable source of energy for these autotrophic forms.

7. Sulphur bacteria contain hydrogen acceptor (in Wieland's sense) and catalase. The mechanism of the sulphur formation and oxidation can be accounted for by Hopkins's glutathione theory.

It is a pleasure to express gratitude to Dr. Flora M. Scott, Dr. Lawrence Irving, and Mr. Lyman Daugherty, M.A., for their kind help in some of the experimental work.

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[1] See for opposite reaction G. H. Drew, Carnegie Publ., lxxxii, 1914.

Hannevart (loc. cit.) found Ca-thiosulphate granules in *Achromatium*. This substance seems to be a storage product, formed from the liquid sulphur, and capable of subsequent oxidation to sulphate.

EXPLANATION OF PLATE XVI.

Illustrating Dr. Baas-Becking's paper on Sulphur Bacteria.

A Zeiss III D stand was used throughout with Leitz compensation ocular and camera. Wratten Panchromatic plates and red filter.

Fig. 1. *Beggiatoa* and *Chromatium* in brine.   × 700.

Fig. 2. *Beggiatoa*, fresh water.   Heated to 80° C.   × 700.

Fig. 3. *Beggiatoa*, freshwater S crystals.   × 700.

Fig. 4. *Beggiatoa*, young filament on black mud.   × 500.

Fig. 5. *Thiospirillum jenense*, Cohn.   × 1,000.

Fig. 6. *Thiospirillum*, heated to 80° C.   × 500.

Fig. 7. *Thiopedia*.   Owen's Lake.   × 700.

Fig. 8. *Lamprocystis roseo-persicina*.   Fresh water.   × 700.

Fig. 9. S-crystals from killed *Lamprocystis* swarmers.   × 700.

Fig. 10. *Thiothrix*.   Fresh water.   × 700.

Fig. 11. S-crystals obtained from *Lamprocystis* extract.   × 700.

Fig. 12. Freshwater *Chromatium* (*C. vinosum* ?).   × 1,000.

Fig. 13. *Chromatium okeni* (brine).   × 700.

Fig. 14. *Chromatium* (brine) killed by heat ; pseudomorphic S-crystals.   × 700.

Fig. 15. *Thiopolycoccus*.   Owen's Lake.   × 300.

Fig 16. *Paramoecium* with CaSO$_4$ crystals at × ×.   The food vacuoles filled with *Lamprocystis*.   × 300.

BAAS-BECKING—SULPHUR BACTERIA.
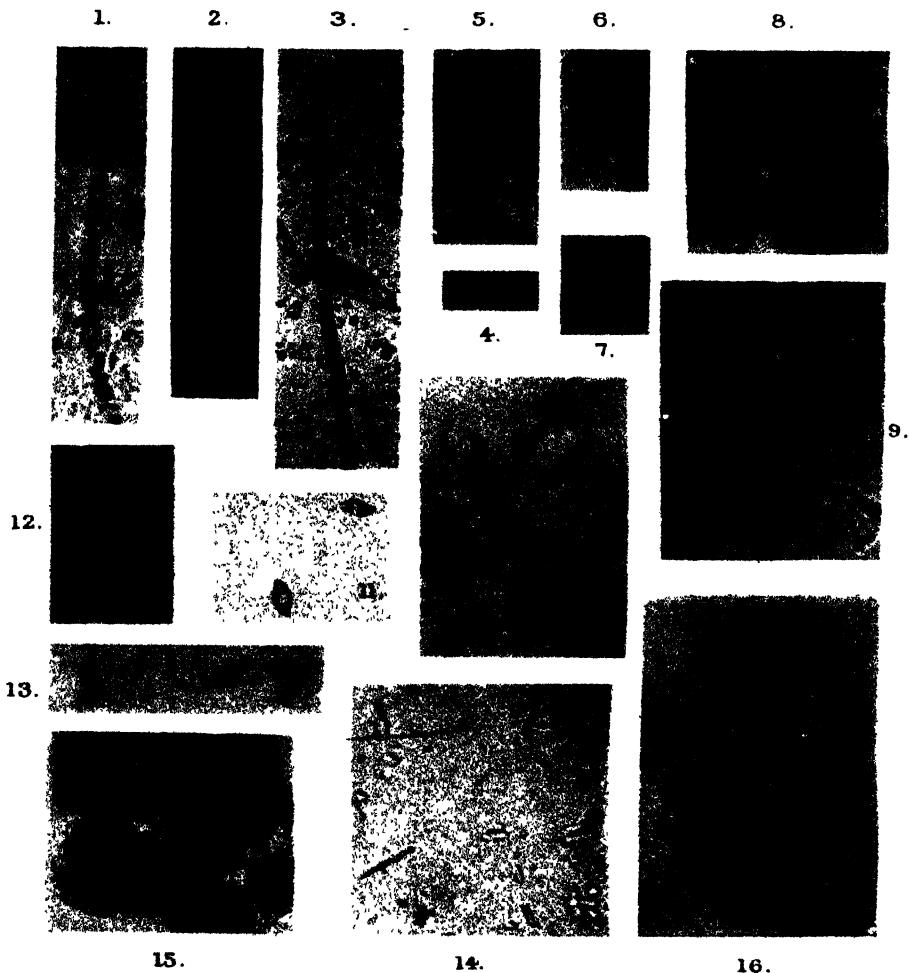
NOTES.

POLARIZED LIGHT AND STARCH GRAINS.—In a paper recently published[1] the claim is put forward that hydrolysis of starch grains by diastase is markedly accelerated by exposure to polarized light. It is suggested, moreover, that a similar effect is probably brought about by exposure to polarized light in absence of the enzyme. Experiments are described in support of these claims and photographs appended to illustrate the appearance of starch grains after exposure to polarized light as compared with that of controls kept in ordinary light or in darkness.

The occurrence of such a phenomenon would have very important bearings on plant physiology; it is most desirable, therefore, that the facts should be established by evidence to which no exception can be taken. It appears to the writer that the evidence that has been put forward is far from being of this character. The reasons for this view are briefly as follows.

When taka-diastase is allowed to act upon whole starch grains at laboratory temperatures, the visible changes induced depend upon differences related to the species of plant from which the starch was derived. In wheat, the grains are lenticular in shape and they become etched by diastase solutions in a very characteristic way. The action usually starts at one point of the periphery, from which an irregular branching channel is formed extending through the body of the grain; later other branching canals may arise, but the number of these is always small, so that even in grains which have suffered considerable disintegration the margin remains for the most part entire. Reference may be made to the figures in Strasburger's text-book and elsewhere. Corrosion occurs, also, most unevenly, intact grains being found alongside others in the last stages of disintegration. Consequently, it is difficult to estimate with any precision the vigour of diastatic activity as a whole from visual inspection of individual grains.

The ovoid starch grains of potato, in the experience of the writer, are much more resistant to the action of diastase than are those of wheat—an observation in agreement with that of Baranetzky, who found that ungelatinized potato starch is highly resistant to the action of diastase, while most of the starches of seeds are readily acted upon. When hydrolytic action of the grain does take place, it is initiated by the removal of a constituent that gives a blue colour with iodine, leaving intact for a much longer time a basis consisting of some other component which does not react with iodine in this way. Since the latter retains the shape of the original grain, the addition of iodine is necessary in order to gain an accurate impression of

[1] E. C. C. Baly, F.R.S., and E. S. Semmens, B.Sc.: The Selective Photochemical Action of Polarized Light. I. Proc. Roy. Soc., B., vol. xcvii, 1925, p. 250.

the extent to which diastatic action has occurred. Reference may be made to Baranetzky's figures, reproduced in Vines' ' Physiology of Plants '.

Turning now to the results presented in the paper under criticism.

The photographs reproduced in Figs. 2, 3, and 7 are referred to as illustrating the effects of hydrolysis obtained in the various experiments described in the text. In respect to these figures the following remarks may be made :

(1) In the experience of the author and of other observers, neither wheat nor potato starch grains show any visible effect from the action of a *strong* diastase solution at laboratory temperatures in so short a time as four hours—the longest time used in these experiments—whether the action takes place in light or darkness. Yet Fig. 2 illustrates the effect of ordinary light, ' there being slight hydrolysis in two of the grains '. In an experiment with potato starch it is recorded that after about an hour there was ' slight hydrolysis in ordinary light '. In all these experiments a weak solution of diastase was used. When wheat starch was exposed to polarized light without the addition of diastase it is recorded that after four hours ' marked hydrolysis ' occurred, while ' a very small effect ' was obtained in ordinary light and no effect in darkness.

(2) The effect figured as due to hydrolysis brought about by exposure to polarized light is quite unlike that ordinarily induced by diastase solutions, which has been described above.

(3) The grains figured provide no proof that hydrolysis has occurred, as similar appearances can be induced by other means. If starch grains mounted in water are subjected to sudden slight pressure, results exactly comparable to those figured can be produced. If starch grains are mounted in a small quantity of liquid under a cover-slip, the latter, unless supported in some way, is liable to exert pressure on the grains as the liquid evaporates. It will be noted in the figures that the formation of a large number of small nicks round the margin of the grains, characteristic of the pressure effect and not at all resembling the usual effect produced by diastase, is most notice-able in the larger grains (which are likely to receive most pressure). Also the ovoid shape of the starch grains of potato tends to assume a more circular outline after pressure.

(4) The photograph reproduced in Fig. 4 is described as showing ' crystals of sugar '. No proof, however, of their chemical nature is offered !

Finally, the writer has endeavoured to repeat the experiments described on p. 251 under conditions such that the grains could not be subjected to accidental pressure. For some of these experiments daylight was used, for others the light from a 100 c.p. pointolite lamp filtered through a water screen was employed. Diastase was supplied in concentrations ranging from 1 per cent. to 0·01 per cent. Exposures to polarized light for twelve hours and longer were given without obtaining the effects described. When eventually some action on the grains became apparent, the appearance was of the usual character induced by diastase solutions bearing no resemblance to grains that had been under pressure. Further, no indication was obtained that the action pro-ceeded more rapidly in the preparation exposed to polarized light than it did in the controls exposed to ordinary light or in darkness.

In a further experiment starch grains (both from potato and wheat) were spread

from a suspension on the surface of a gelatin plate containing diastase. Different areas of the plate were illuminated with polarized light, ordinary light, or unilluminated. After a length of time determined by trial, the plate was treated with iodine and examined microscopically. No different appearance of the grains was observed, while a uniform colour was given over the whole plate, there being no suggestion of one area being bluer and another redder, as would have been expected had there been any differential rate in the action of the diastase. Similar failure to obtain any differential effect resulted when gelatinized starch was used.

In conclusion, it may be said that the nature of the evidence offered in the paper under criticism, the negative results obtained on repeating under more critical conditions some of the experiments described in that paper, and the failure to obtain positive results in the further experiments referred to above, lead to the view that, whatever may be the specific effects induced by polarized light upon starch grains, more critical evidence is required before concluding that hydrolysis is among them.

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SPORANGIA AND THE FORMATION OF COLONIES IN CHLAMYDO-MONADALES.—That an apparently important structural difference may depend upon a slight variation in development is illustrated by a comparison of the genera *Sphaerella* and *Stephanosphaera*. The cells of the latter differ from those of the former inasmuch as they remain enclosed throughout the motile vegetative period within the sporangial wall. In such an example there is very little essential morphological distinction between a purely unicellular type and a many-celled colonial type, and the two genera belong to the same family without being connected through fewer celled colonial types.

Divisions within the asexual sporangium [1] of *Sphaerella* result in the formation of 4 to 64 offspring cells, the zoospores. The convenient terms megazoospores and microzoospores have been used [2] to distinguish the larger and smaller kinds, but there is a gradual transition, the intermediate numbers of 8, 16, and 32 zoospores being formed in some sporangia.[3] In the Chlamydomonadaceae and Carteriaceae, 2, 4, or 8 zoospores are normally formed in each parent cell. These families, therefore, only possess megazoospores. Even *Chlorogonium*, probably belonging to the Sphaerellaceae,[4] must be included in this statement. On the other hand, a higher number of division products is, of course, found in the sporangium of all the larger colonial Chlamydomonads, these genera having nothing to correspond with a megazoospore.

The occurrence of microzoospores in *Sphaerella* shows that the tendency to produce relatively high numbers of offspring cells may exist independently of the colonial habit. Division of such a sporangium cannot, of course, be compared with

[1] The gametangium of *Sphaerella* may show 128 cells ; of *Chlamydomonas*, 32 to 64 cells.
[2] West, G. S.: Algae. Cambridge Botanical Handbooks. I. Cambridge, 1916.
[3] loc. cit. [4] loc. cit.

the complete process of segmentation of the asexual reproductive cell of *Volvox*. Nevertheless, a mechanism capable, with slight modifications, of producing 32- or 64-celled colonies already exists in *Sphaerella*. Falkenberg [1] actually compared the whole colony of *Volvox* with the free contents of a sporangium as in *Hydrodictyon* and *Chlamydomonas*, a view which has been adversely criticized by Harper,[2] who, whilst agreeing that *Volvox* retains the multiple or colony reproduction as in *Sphaerella*, points out that there is the additional feature of growth regularly alternating with cell-division, except perhaps in the first few divisions. In this respect the development of the coenobium of *Volvox* is comparable with that of the body of one of the higher metaphytes.[3] There is, however, greater similarity between the sporangial type of division and that seen in *Volvox*. In both the latter cases cell-division results in a great reduction in size of the cells. In the bodies of the higher Metaphyta, on the other hand, each cell attains the size of its parents before it divides again. Such metaphytic bodies are termed pletheas by Janet,[4] who makes a fundamental distinction between these and what he terms blasteas, namely bodies which are segmented into a number of cells before the chief growth in volume takes place. Both a sporangium and a coenobium would be classed as blasteas.

A simple type of plethea is found, for example, in the filament of *Ulothrix*. There is nothing to show that a multicellular plant body of this kind has been derived from a blastea; rather the two types of structure appear to have originated side by side, and they represent two distinct stages in the ontogenesis of most organisms. Janet [5] compares the typical plethea with the phase in the life-history of a flagellate when binary fission, alternating with growth to the size of the parent cell, occurs. This latter phase, in the flagellates, he classes as a special kind of plethea, the sporadea, and distinguishes it from the sporangial stage, which is a blastea.

Division of the cells beyond the four-celled phase has been observed by Chodat [6] in certain variants of *Chlamydomonas* which he calls 'états larvaires'. Here the division is not always regular, but the resulting forms approach 8- and 16-celled colonies of *Gonium*. On the other hand, sporangial division in *Sphaerella*, frequently resulting in 32 or 64 offspring cells, agrees with that of *Eudorina* or *Pleodorina*.

The view of the origin of *Volvox* from ancestors of the *Sphaerella* type [7] implies that the protoplasmic connexions are an inheritance from the unicellular stage. Hence they may be expected to appear in the earliest stages of individual development. Janet [8] concludes from the form and arrangement of the 4-celled and even the 2-celled colonies of *Volvox globator* (L.), Ehrenb., and *Janetosphaera aureus* (Ehrenb.), Shaw (= *Volvox aureus*, Ehrenb.), that they are represented in these

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[1] Falkenberg, P.: Die Algen im weitesten Sinne. Schenk's Handbuch der Botanik, Encyklopaedie der Naturwissenschaften, i. 23. Breslau, 1881.

[2] Harper, R. A.: Binary Fission and Surface Tension in the Development of the Colony of *Volvox*. Brooklyn Botanic Garden Memoirs, i, 1918.

[3] loc. cit.

[4] Janet, C.: Le Volvox, Deuxième Mémoire. Paris, 1922.

[5] loc. cit.

[6] Chodat, R.: Algues vertes de la Suisse. Matér. Flore Crypt. Suisse, i, 1902.

[7] Crow, W. B.: The Classification of some Colonial Chlamydomonads. New Phytol., xvii, 1918.

[8] Janet, C.: Le Volvox. Limoges, 1912.

earliest stages. They are clearly visible as soon as the cells become separated. As in *Sphaerella* they are left as bridges to the outer cell membrane when the latter becomes detached from the protoplast by the development of the inner mucilage layer.

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February 1925.

' EQUISETOID ' HAIRS.—M. Renault has figured certain curious hairs which he found on the surface of the stems and leaves of *Botryopteris forensis.* These hairs, as described by Professor Bower from slides belonging to the late Dr. Kidston, are
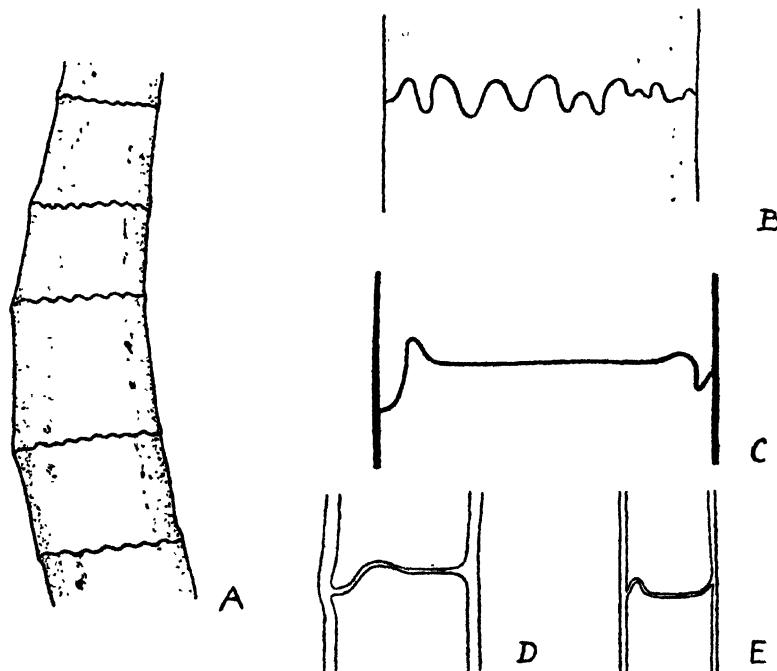
Hairs of *Dicksonia squarrosa*. A, base of hair (× 65) ; B, junction between two cells of the hair seen in surface view ; C, the same in optical section ; D and E, actual longitudinal sections of more distal portions of a hair (B, C, D, and E × 320).

' relatively large stiff hairs, transversely septate, and each is seated on a multi-cellular emergence projecting from the surface of the petiole '. The earlier accounts. as for instance that of Scott,[1] stated that ' each cell bore a ring of teeth at its distal end, giving the hair the appearance of a miniature *Equisetum* '. Hence the description of them as ' equisetoid' hairs.

[1] Scott, Studies in Fossil Botany, p. 236.

Professor Bower [1] has recently demonstrated that the 'equisetoid' appearance is due, not to a ring of teeth at the distal end of each cell, but to the fact that 'the margins of the transverse septa are thrown i·to deep and rather regular corrugations, with the result that upturned processes o. equivalent size, each appearing to have protoplasmic contents, are seen at the uy·)er limit of each lower cell just below the septum, but not segmented off from it. These processes interlock with similar downward processes from the upper cell. Each septum of the hair being thus frilled, their characteristic appearance follows.'

Such 'equisetoid' hairs have not been described hitherto in any of the living Ferns. An examination of *Dicksonia squarrosa*, Swartz, and *Blumei*, Moore, has shown, however, that the hairs borne on the petioles of these species present the 'equisetoid' appearance. The hairs differ from those of *Botryopteris* in being simple, though the basal cells are much enlarged (A). As seen in surface view each cell of the hair appears to bear a ring of rather irregular teeth (A and B). In section, however, the hairs present the appearance shown in C, D, and E, indicating that the 'equisetoid' appearance here, as in *Botryopteris forensis*, is due to a frilling of the peripheral regions of the transverse septa, the outer wall being smooth and cylindrical.

Stiff hairs on the rhizome of *Metaxya rostrata* show exactly the same features as those above described; so it appears that the presence of these peculiar hairs is not confined to any one circle of affinity.

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[1] Bower, The Ferns, vol. i, p. 199.

The Anatomy and the Morphology of the Flower of Euphorbia.

BY

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With one hundred and twelve Figures in the Text.

THE affinities of the Euphorbiaceae are extremely doubtful, though modern botanists place the family in the Geraniales. This position is based on the orientation of the ovules and on the ventral position of the raphe. In other respects the family shows no surviving features in common with other members of the alliance. In its grosser structure the genus *Euphorbia* departs from other genera of the family because of reduction, which makes it difficult to determine the true morphology of the floral parts. This condition has led to many interpretations of the nature of the inflorescence and the flower. But it is impossible to determine the phylogenetic position of any group without a thorough morphological knowledge of its component members, particularly its flowers. Since *Euphorbia* possesses many characters which may have arisen through ecological changes, another clue to the definite morphological nature of structures is that afforded by internal anatomy. This particularly includes the skeletal system, which is a very conservative part of plant structure. By this means morphological evidence regarding the nature of the inflorescence and its flowers may be strengthened, so that there may be more definite knowledge upon which phylogenetic relationships may be based. Some of the old evidence concerning the nature of the flower of *Euphorbia* will be reviewed and new brought forward.

THE MORPHOLOGY OF THE GENUS *EUPHORBIA.*

The genus *Euphorbia*, the largest of the family Euphorbiaceae, exhibits much uniformity in its general morphology, but great diversity in its more detailed characters. It includes trees (*E. pulcherrima*) which

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attain considerable height, shrubs (*E. portulacoides*) of various habitats, and prostrate (*E. hirsuta*) as well as erect herbs (*E. corollata*). The stems may be slender and wiry (*E. hexagona*) ; thickened, cactus-like (*E. splendens*) ; or very leafy (*E. Cyparissias*). Usually the leaves are entire, of opposite or alternate phyllotaxy. The inflorescence is more or less compound, and consists of cymose and dichotomous branches which are terminated by cyathia, the so-called ' flowers ' of the *Euphorbia*. The outer cup-shaped, five-lobed, gamophyllous structure of the cyathium is the involucre (Fig. 6, *b*). Its divisions may be entire, cleft, or laciniate. The sinuses of the involucre bear large thick glands (Fig. 6, *g*), which are sessile, or stalked (Fig. 1, *g*). In some species they are accompanied by petaloid appendages (Fig. 1, *a*), while in other species they are absent (Fig. 5). The glands vary in number ; five seeming to predominate, although four are found almost as frequently. In such a case the fifth sinus then serves to lodge the reflexed peduncle of the pistillate flower (Fig. 6, *P*). These glands may be entire or bi-cornute (Fig. 5, *g*) in their outlines. A few species representative of the group *Poinsettia* bear but a single enlarged gland without petaloid appendages (Fig. 2, *g*), the remaining four having become abortive or entirely lost.

Each lobe of the involucre, which may be interpreted as a single bract, subtends a group of monandrous flowers (Fig. 3) [1] which are arranged in definite sequence, and are to be discussed subsequently. The flowers develop in centrifugal order, those nearest the cyathium axis developing first (Fig. 3, *F*). Each monandrous flower in reality is but a single stamen (Fig. 4, *s*), joined to a pedicel *h*, the top of which represents the receptacle of the flower. A slight circular articulation *G* occurs just above the pedicel of each monandrous flower, that is, between the pedicel and its respective filament. In many species these monandrous flowers are borne in the axil of minute bracts, which may enclose the entire staminate flower at its base (Figs. 100, 101), or may appear only on two sides of it (Figs. 103, 104), or frequently may be vestigial or entirely absent (Fig. 111). When present, the distal end of the bract may be entire, dissected, or finely fimbriated, so as to intertwine among the stamens (Fig. 104, *z*).

The solitary pistillate flower (Fig. 2, *P*) terminates the axis of the

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[1] Throughout this paper, the following letters indicate the similar structures in all illustrations :

a, petaloid appendage ; *A*, large scarlet bracts of *E. splendens* ; *b*, bract ; *B*, bracts of suppressed branches ; *c*, involucral commissure ; *d*, disc ; *D*, oldest monandrous flower group, *e*, branch trace components ; *E*, stele of suppressed branches ; *f*, foliar gap ; *F*, youngest monandrous flower ; *g*, gland ; *G*, articulation ; *h*, pedicel of monandrous flower ; *i*, stele of inflorescence ; *j*, dorsal carpellary trace ; *k*, ventral carpellary trace ; *L*, left branch of dichasium ; *m*, monandrous flower ; *n*, median line of gland ; *o*, ovulary traces ; *p*, primary axis ; *q*, carpellary trace ; *R*, right branch of dichasium ; *s*, stamen ; *t*, tertiary branch ; *u*, style ; *v*, vestigial steles ; *w*, stigma ; *x*, xylem ; *y*, phloem ; *z*, bracteole ; *P*, pistillate flower.
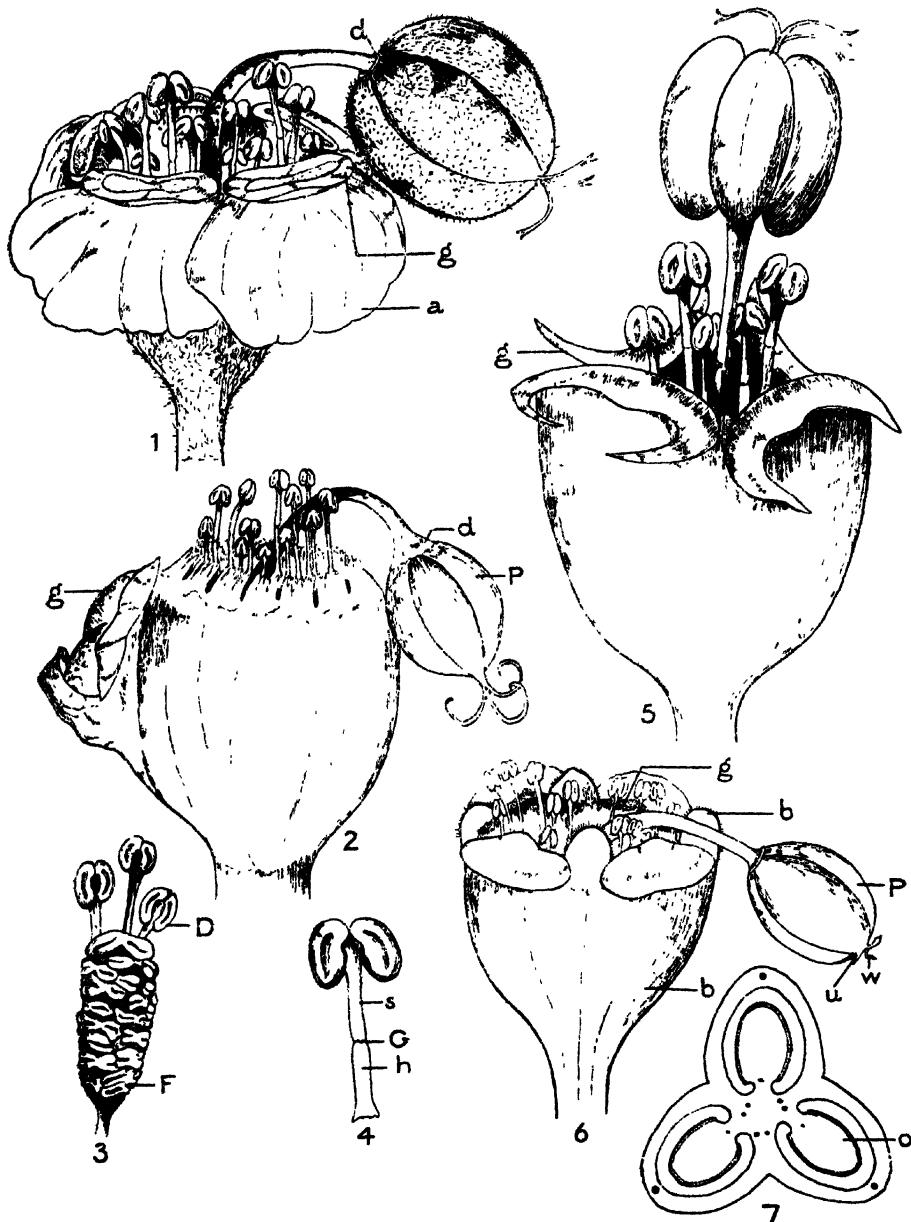
FIGS. 1–7. 1. *Euphorbia marginata* : entire cyathium. 2. *E. pulcherrima.* 3. *E. pulcherrima* : a single group of monandrous flowers of the cyathium. 4. *E. pulcherrima* : a single monandrous flower. 5. *E. Esula.* 6. *E. clusiaefolia.* 7. *E. clusiaefolia* : transverse section of a pistil to show axial placenta.

condensed inflorescence within the involucre. By means of its long pedicel it protrudes beyond the upper level of the stamens. Apparently it is achlamydeous, but the perianth is represented by an entire (Fig. 1, *d*) or, more or less, three-lobed hypogynous disc. The ovary consists of three carpels usually united, rarely free ; entire or two-valved. In the inner angle of each loculus is an axile placenta (Fig. 7), supporting an anatropous descending ovule, with ventral raphe and superior exterior micropyle, capped by an obturator. The latter is a mass of variable form springing from the placenta, like a second ovule superposed to the first.

The pistil bears one to three styles, which are short (Fig. 6, *u*). The stigma (Fig. 6, *w*) generally equals the carpels in number, though it may become dissected, fimbriated, flat, fringed, or marked on the inner surface by a deep ridge. Upon maturity the pistil forms a capsule with three loculi, in each of which is suspended a single seed possessing three integuments : a cellulose exterior, a middle testaceous, and an inner membranaceous one.

The uncertainty of the systematic position of this family largely may be attributed to the diverse interpretations of the morphology of the inflorescence, and of its parts.

From the time of Linnaeus (1753) (45), almost to the present, two conflicting theories seem to have reigned in botanical circles. The one, and the older view, recorded in the works of Linnaeus, maintains that the *Euphorbia* 'flower' is a true flower, polyandrous and hermaphroditic, with a distinct calyx. Adanson (1763) (1), Lamarck (1786) (42), and A. de Jussieu (1824) (38) each stated this conclusion. It was not until almost a half-century later that R. Brown (1818) (18) worked out the true morphology of this deceptive inflorescence. He asserts that the 'flower' is an androgynous inflorescence. The ovary is a pistillate flower, and the staminate flowers, reduced to stamens only, form lateral inflorescences, arising in the axil of peripheral leaves, serving as involucral bracts, not sepals. In his later years Roeper (1824) (59), and even Wydler (1845) (76), sanctioned this new conception. On the other hand, these views were quite repugnant to such botanists as Payer (1857) (53) and Baillon (1858) (3), whose investigations led them to support the fundamental ideas of Linnaeus. By this time the status of the flower was far from an established fact. Beginning with de Candolle (1866) (23) and followed later by Müller (1872) (48), Warming (1870) (69), Schmitz (1871) (63), Hieronymus (1872) (36), Bentham and Hooker (1883) (7), Čelakovsky (1872) (21), and Van Tieghem (1875) (68), the general interpretation by R. Brown seems to have been accepted with numerous minor changes. Thus much evidence has been deduced from ontogeny, morphology, and teratology in an effort satisfactorily to explain the 'flower' of *Euphorbia*. As a result all controversies have led to two conflicting statements. The

first implies that the *Euphorbia* flower is hermaphroditic ; the second, that the flower is a condensed inflorescence. The latter view seems, however, to have a greater number of adherents among modern taxonomists.

THE OBJECT OF THIS INVESTIGATION.

These two opinions regarding the morphology of the flower seem to have been thoroughly verified, each by its own supporters, but mostly from the fields of morphology and ontogeny. It has been suggested that probably the anatomy of the floral structures would give more positive proof of their detailed morphology, supporting one or the other fact, and thus aid in a possible determination of the phylogenetic position of this genus and the family. With this basic aim, the flowers of thirty-three species of *Euphorbia* were studied. This selection included representatives not only from different parts of the same plant, but from different plants representing widely separated habitats. The list includes plants from various habitats in both Eastern and Western Hemispheres ; six species indigenous to the Tropics, sixteen to the North Temperate Zone, and two to the South Temperate Zone.

TECHNIQUE.

The inflorescences from living plants were killed in 70 per cent. alcohol, and serial microtome sections were made in both transverse and longitudinal planes from specimens embedded in paraffin. Herbarium material was soaked in distilled water overnight ; then boiled for five minutes in a 2 per cent. solution of potassium hydroxide, washed for ten hours in running water, dehydrated and embedded as usual. For staining, a 1 per cent. aqueous solution of crystal violet and a $\frac{1}{2}$ per cent. solution of erythrosin in clove oil proved most satisfactory. With these stains the xylem was coloured clear blue, the phloem purple, and cutinized areas a very dark blue, while remaining tissues became pink.

DESCRIPTION OF SPECIES.

The general morphology based upon anatomical studies may best be presented by discussing a few species, the anatomical characters of which represent certain types, portraying primitive, as well as specialized features.

The twenty-eight species studied in detail may be divided into three groups. The first group suggests the more primitive conditions ; the second, the more specialized features of the genus ; the third represents a transitional form. Though some of the members of Group I give a clue to the probable ancestral conditions, a few species like *E. pulcherrima* and *E. dentata* have progressed more rapidly, as suggested by the reduction and the elaboration of the glands.

GROUP I.

*1. *E. clusiaefolia*, Hook. & Arn.
*2. *E. dioscoreoides*, Boiss.
*3. *E. portulacoides*, Spreng.
*4. *E. pulcherrima*, Willd.
*5. *E. dentata*, Michx.
*6. *E. marginata*, Purch.
7. *E. fulgens*, Karw.
8. *E. insularis*, Boiss.
*9. *E. buxifolia*, Lam.
*10. *E. crenulatus*, Engelm.
*11. *E. pilulifera*, L.
*12. *E. splendens*, Bojer.

GROUP II.

*28. *E. corollata*, L.

GROUP III.

13. *E. stricta*, L.
*14. *E. cordifolia*, Ell.
*15. *E. Palmeri*, Engelm.
*16. *E. Preslii*, Guss.
17. *E. lucida*, Waldst. & Kit.
18. *E. commutata*, Engelm.
19. *E. petaloidea*, Engelm.
*20. *E. Darlingtonii*, Gray.
*21. *E. Helioscopia*, L.
*22. *E. calcicola*, Fernald.
*23. *E. arkansana*, Engelm. & Gray.
*24. *E. Cyparissias*, L.
*25. *E. hirsuta*, (Torr.) Wiegand.
*26. *E. albomarginata*, T. & G.
*27. *E. Esula*, L.

The * (asterisk) indicates those species the pistillate flowers of which were observed in detail.

The external features of the following species were observed, but it was impossible to make serial sections by the methods which were employed :

1. *E. Fendleri*, T. & G.
2. *E. eriantha*, Benth.
3. *E. polycarpa*, (Benth.) Millsp.
4. *E. peplus*, L.
5. *E. hexagona*, Nutt.

E. clusiaefolia, Hook. & Arn.

This species will suffice in general as a standard in noting variations. The axial cylinder of the peduncle of the flower is represented by a series of six highly specialized steles, one of which occupies the centre of the stem, and the remaining five arranged in circular fashion about the central one which is the main axis of the cyathium (Fig. 8). Each of the five smaller steles contains several strands of xylem x arranged in a ring, embedded in parenchyma and encircled more or less by phloem y. The xylem of the central stele is less uniformly distributed, but is mainly in three large groups q. These latter strands form the supply to the pistil. At a higher level (Fig. 9) each of the five outer steles moves slightly into the cortex. This outward migration of each stele is accompanied by radial chorisis of the xylem, which aids in increasing the diameter of the stele.
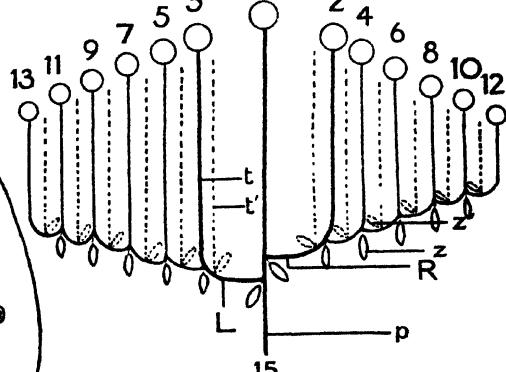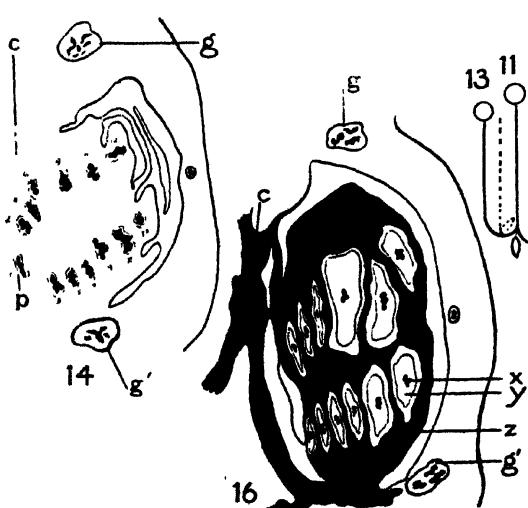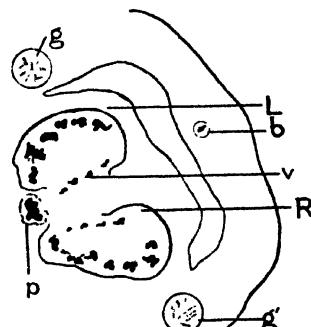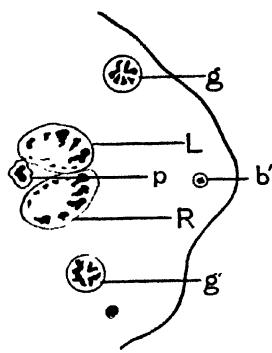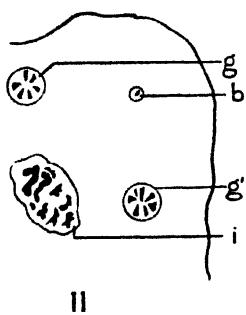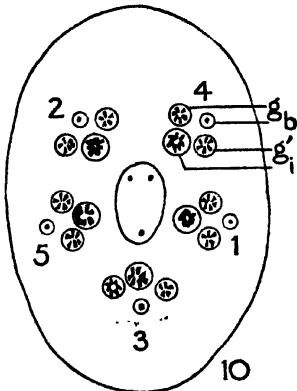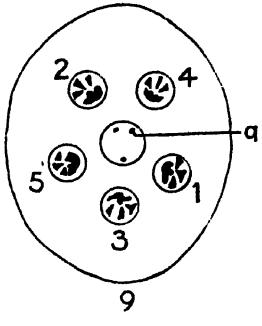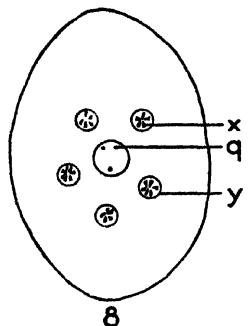
FIGS. 8-16. *E. clusiaefolia.* 8-14. Transverse sections of the peduncle showing the behaviour of the primary and pistillate steles. 15. Diagrammatic median longitudinal section showing the course of the vascular bundles in a lateral inflorescence. 16. Transverse section of a single inflorescence ; pedicels of stamens surrounded by bracteoles.

The outward migration of the five primary steles of the peduncle does not take place simultaneously, but occurs in such a way as to effect a spiral distribution. Beginning with a primary stele as No. 1, and following the others in clockwise or counter-clockwise succession, the various steles are numbered according to their ontogenetic sequence, namely 1, 4, 2, 5, 3 (Fig. 9).

For convenience, the further behaviour of only one of these primary steles will be discussed. All others excepting the central one are similar. The central stele or main axis will be considered later. Each of these primary steles reorganizes so that instead of one primary stele, simultaneously four masses are formed (Fig. 10, Group 4). Three of these four masses are steles, while the fourth and smallest mass is a single leaf trace. The three outermost xylem masses supply the involucral bract and gland. The smallest of the three masses with its weakly developed xylem functions as the trace of the bract (Fig. 10, *b*). The two steles *g*, *g′* of this outermost group resemble highly specialized branches whose two original traces have become fused. These siphonosteles *g*, *g′* may be considered the first or basal secondary branches arising from the primary lateral axis *i*. The remaining or fourth mass *i*, the primary axis, is the most important of all. This is the continuation of the main axis, and therefore is the primary axis which supplies the floral branches and their respective flowers, that is, one of the main lateral branches of the inflorescences. This part of the inflorescence consequently arises in the axil of the involucral bract *b*; its basal branches *g*, *g′* arise very near to its point of origin. The three outer vascular masses gradually depart from the continued primary lateral, axis *i* by passing upward and outward towards the periphery of the stem.

Coincident with this migration is the enlargement by chorisis of the primary siphonostele (Fig. 11, *i*), this resulting in the rapid proliferation of the xylem elements. Eventually there are three groups of xylem strands, two large stele-like masses (Fig. 12, *L*, *R*) and a third solitary strand (Fig. 12, *p*). These steles form the second pair of secondary steles derived from the primary axis. They are in juxtaposition, their greatest diameter in a radial plane. The third mass (Fig. 12, *p*), which represents the further continuation of the primary axis, is a small concentric strand wedged between the inner margins of the secondary siphonosteles. The conducting tissue in each of these large secondary steles is so organized that there is a predominance of xylem elements towards the margin nearest the involucre, and smaller, less-lignified masses towards the portion of the periphery nearer the primary axis (Fig. 12, *p*). Thus these two steles take the outline of two large horseshoe-shaped masses (Fig. 13), the outer halves of which contain the stronger and greater number of xylem elements ; the inner halves, the much weaker and fewer strands (Fig. 13, *v*). These latter

strands play no rôle whatever in supplying floral organs, for they soon end abruptly and thus clearly represent lost or abortive branches. The behaviour of these large steles (Fig. 13, *R, L*) leads to the conclusion that each horseshoe-shaped mass represents a principal secondary branch of the primary axis (Fig. 13, *p*). This reorganization of steles forms a type of inflorescence called a dichasium ; the primary axis of which bears these two important secondary branches (Fig. 13, *R, L*).

At this level the involucral bract, supported by three vascular strands (Fig. 13, *g, b, g'*), severs itself from the floral mass, excepting in the region where the lateral edges of bracts have coalesced to form an involucral partition (Fig. 13, *c*). By the gradual disappearance of the abortive xylem strands (Fig. 14) the floral vascular supply is composed of but two radial rows of primary xylem, the proximal ends of which almost meet the xylem of the primary axis (Fig. 14, *p*). Each row is composed of six strands of vascular tissue, which are arranged alternately with the strands of the opposite row of the same inflorescence. The innermost branch (Fig. 14: 2 or 3) of each row represents the secondary or lateral branch of the primary axis (Fig. 14, *p*). Each of these secondary branches contains five smaller tertiary branches (Fig. 14: 4, 6, 8, 10, 12), the cluster of xylem destined to supply each ramus arising abruptly from the preceding branch. This produces another type of inflorescence, a monochasium, in which each relatively main axis bears one lateral branch. Hence growth is centrifugal. The oldest and longest branch (Fig. 14: 2 or 3) is nearest the centre of the entire cyathium, while the youngest and shortest branch is in close proximity to the involucral bract of that group or inflorescence (Fig. 14: 13).

Previous to this development of tertiary branches, the arrangement of rami has been of the dichasial type (Fig. 15: 1, 2, 3). This arrangement then becomes supplanted by a monochasium due to the abortion and complete loss of the inner lateral branch of each relatively main axis. The behaviour of the vascular tissue indicates that the inner branches, represented only by the small indefinite branches of xylem in the secondary stele, are lost. Fundamentally each branch should be subtended by a single bracteole. The bracteoles of the developed tertiary branches are present.[1] These subsequently will be discussed. The bracteoles of the abortive branches have disappeared, together with their respective axes. Fig. 15 illustrates diagrammatically the vascular supply to the floral organs, the dotted lines indicating the abortive tertiary branches *t'* with their respective bracteoles *z'*. The continuous lines represent the existing tertiary branches *t* and their bracteoles *z*. Numerals indicate the order of divergence of the branches with their respective flowers.

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[1] Solid black areas indicate the bracteoles of the tertiary branches whether they arise from the base of the branches or from the involucral commissure, whether they be fused one to another or distinctly segregated.

Each xylem group (Fig. 16, *x*) is destined to supply a branch or pedicel which bears at its terminus a single monandrous flower. The vessels pass uninterrupted through the articulation which delimits filament and pedicel, on into the filament itself. At the level indicated by Fig. 17, the pedicels of the stamens are completely surrounded by parenchymatous tissue (black areas) which represents the bases of bracteoles *z* fused to the various tertiary branches. Their vascular supply has become lost. Bracteoles are fused to one another to form the involucral partitions or commissures (Figs. 13, 14, 16, *c*). But at a level about two-thirds the length of the involucre, the distal parts of the bracts become very finely laciniate, so that finally the stamens become lost in a mass of slender threads (Fig. 18). Each staminate flower is a single stamen, terminal on the pedicel and distinguishable externally from a stamen only by a slight joint or articulation which marks the insertion of the filament upon the pedicel.

In the meantime the lateral traces of the involucral bract which have become entirely free from the floral mass undergo change (Fig. 17, *g*, *g'*). Each mass of xylem in the lateral trace increases very rapidly in a tangential plane by means of radial chorisis. This continues till the vascular elements of the lateral trace of one bract (Fig. 18, *g*) become contiguous with the vascular elements of the lateral trace of the adjacent bract (Fig. 18, *g'*), so as to form a tangential band of vascular tissue between and opposite two branches of the inflorescence (Fig. 19, *g*, *g'*), excepting between branches 2 and 5 (Fig. 19, *g''*, *g'''*). Immediately these vascular tissues, as a result of tangential division for their entire length, send off branches *a*. These branches later supply the gland. These organs alternate with the involucral bracts. Since the gland is supplied with strands which originate at the primary axis and traverse the involucre, this organ is to be considered an appendage of the primary axis.

Fig. 20 diagrammatically recapitulates the entire vascular system of the cyathium, exclusive of the pistillate flower. Nos. 1 to 5 represent the several primary axes, each of which sends off one leaf trace *b* to supply the involucre, and two secondary branches *g*, *g'* which pass through the involucre to supply the gland. A second pair of secondary branches *L*, *R* arises from the main axis at *p*. Each of these branches then gives rise to five tertiary branches (4, 6, 8, 10, 12, or 5, 7, 9, 11, 13) which form a monochasium.

The vascular tissue of the central stele, which in the peduncle of the cyathium is composed of three strands (Fig. 21), continues its upward course for a considerable distance without reorganization. It eventually supplies the pistil. At the same level as that at which individual branches are separated from their respective bracteoles, the stele of the pistil also becomes separated from the common involucral partitions and adjacent bracteoles (Fig. 17). The syncarpous pistil is composed of three carpels
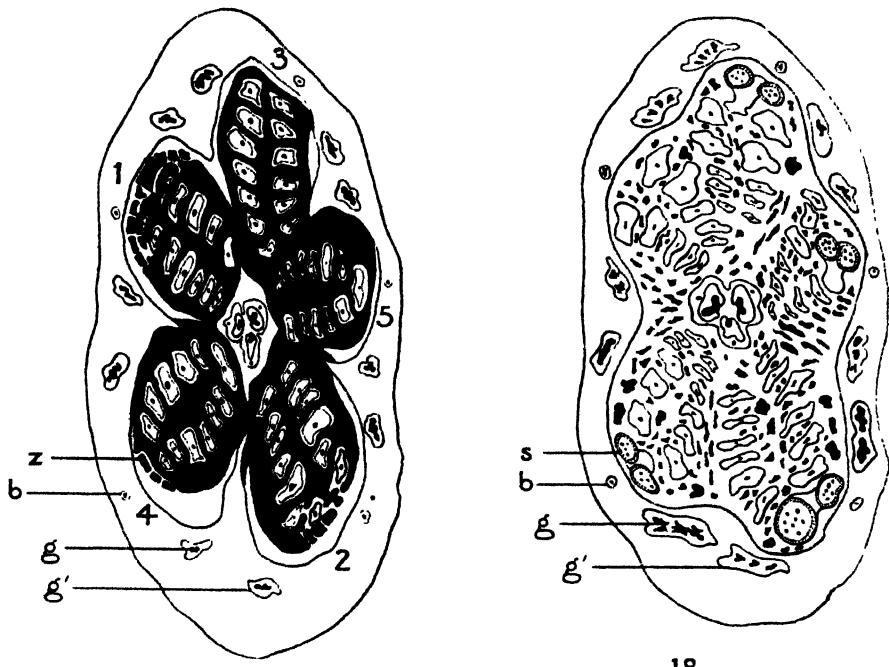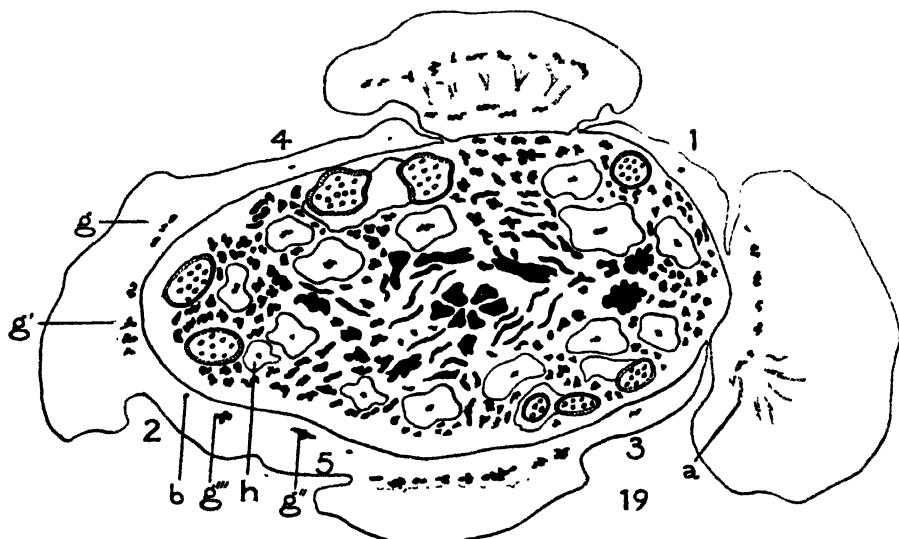
FIGS. 17–19. *E. clusiaefolia.* Transverse sections of the cyathium. 17. Pedicels of stamens surrounded by bracteoles; increase of vascular supply in the involucre. 18. Stamens free, bracteoles fimbriated, steles of gland enlarging. 19. Glands differentiated, oldest stamens evident, bracteoles fimbriated, stigmas free.
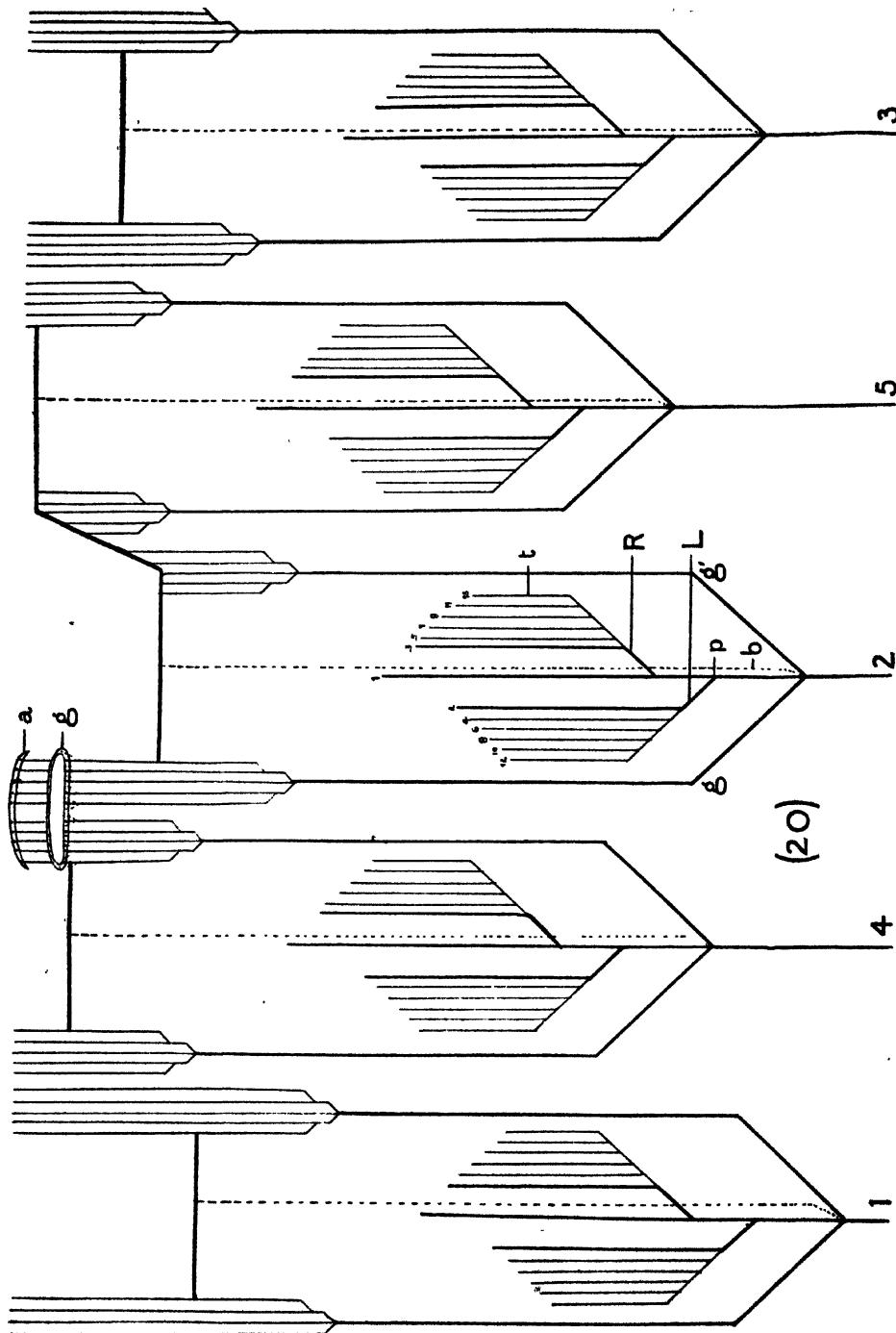
FIG. 20. *E. clusiaefolia.* Diagram of the entire vascular system of the cyathium exclusive of that of the pistillate flower. The 'flower' is split down one side and laid out in one plane.

situated upon a slight rim. Each carpel is supplied by a single strand of vascular tissue (Fig. 22, *q*). The axial bundle radially segments into three strands, a median and two lateral traces. The median or dorsal trace (Fig. 23, *j*) to each carpel arises first (Fig. 22, *j*), departs from the axis, and passes upward along the dorsal margin of the carpel into the style and stigma. There it gradually fades out (Fig. 30, *j*). At a level on the axial cylinder, just above the point of departure of the dorsal trace, each of the two axial strands which have resulted from the origin and departure of the dorsal carpel trace forms a ventral trace (Figs. 23, 30, *k*). The ventral traces migrate along the ventral suture of the carpel and continue their
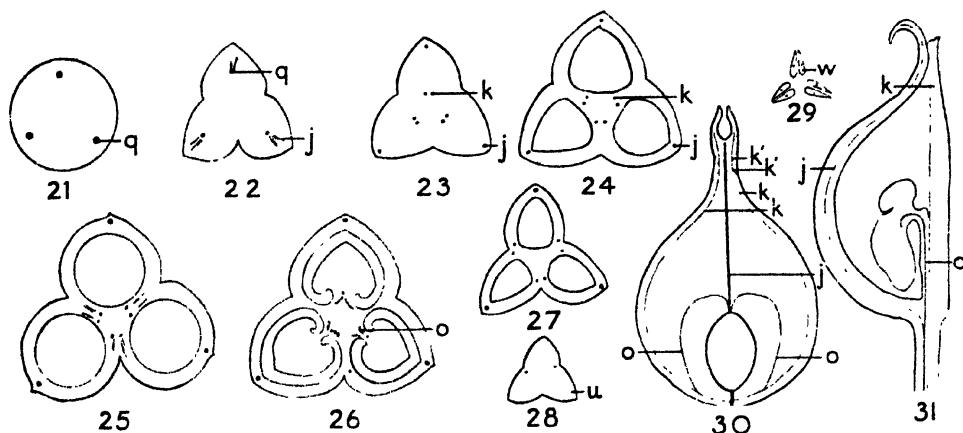
FIGS. 21–31. *E. clusiaefolia.* Diagrams illustrating the mode of origin, course and orientation of the vascular supply of the pistillate flower. 30. A single carpel split along the ventral suture and laid flat. 31. A median longitudinal section through a single carpel.

upward course through the style (Fig. 30, *k*). In the upper end of the latter two ventral carpellary traces, one from each of two adjacent carpels (Fig. 30, *k*, *k'*), unite only to divide again into numerous strands in the stigma. In the lower portion of the ventral supply course where it departs from the main axis, inwardly each trace gives off a branch *o*, the two strands uniting and supplying the single anatropous ovule, which is abortive (Fig. 30, *o*). Beneath the ovary the pistillate flower bears an abortive perianth in the form of a slight disc or rim, the vascular supply of which is lacking.

At its origin or point of insertion upon the main axis, each bract is subtended by a single foliar trace, and is further supported by two branch traces. The bracts of the gamophyllous involucre become free at the level at which the glands originate. Coincident with this separation is the division of the foliar trace into three bundles, which are distributed through

the remainder of the bract. Thus the three traces characteristic of the leaf have become fused basally and for a considerable distance towards their distal ends.

The so-called 'flower' or cyathium is essentially a condensed inflorescence, suppressed in a gamophyllous involucre of five bracts. Each bract is supported by three traces, its own subtended foliar trace and two others which are decidedly stelar in nature, and eventually ramify to and through the gland. This inflorescence, which is cymose, is composed of a main or central axis and five spirally arranged, sub-whorled, axillary branches, each of which is subtended by a bract. The cyme terminates in a naked pistillate flower which possesses an abortive perianth (with no evidence of vascular supply) in the form of a slight rim below the ovary. Each carpel possesses the normal three traces. Each axillary shoot is, at the base, in the form of a dichasium, the terminal flower of which is represented by a single stamen. The presence and the behaviour of other steles indicate that each of the lateral shoots of the dichasium develops a monochasium of monandrous flowers, each subtended by an abortive bracteole without vascular supply. A slight ring-like indentation below the anther represents the position of an abortive perianth.

E. dioscoreoides, Boiss.

In this tropical species the variations from *E. clusiaefolia* have their origin in the order of departure of the first secondary traces from the primary axis and in the behaviour of the bract trace. After the passing out of the left one of these secondary branches, which forms part of the glandular supply, the gap soon is obliterated by the massing of the remaining vascular tissue. The right secondary branch then severs its connexion with this irregular mass or stele and moves outward through the cortex to the gland, where it breaks up into many small branches.

Simultaneously with the breaking up of the original branch trace is the division of the involucral trace into a right and a left segment. These segments then pass gradually to the outer margins of the involucral bract, where they form the lateral leaf bundles. The small median trace retains its median position. Therefore at its base the apparent single involucral trace seems to represent the fusion of three foliar traces which become distinct in the upper portion of the bract. Except in four species, this feature is characteristic of all species which have been observed.

The lateral bundles of two adjacent bracts, together with the rami of the original branch traces, traverse the three-lobed petaloid appendage, leaving but the median foliar bundle as the supply to the bract. Thus, each bract morphologically is supplied by three traces ; each gland is supported by the numerous branches of two adjacent branch traces and by

two lateral foliar traces, one foliar trace and one branch trace from each of two adjacent primary steles.

After the departure of these branch traces from their respective primary axes, the resultant broken primary siphonostele resumes its continuity and the residual vascular elements reorganize into thirteen uniform groups, each representing a single trace to a monandrous flower. The greater portion of the bracteoles have disappeared, except a few vestiges the bases of which have fused to form a slight involucral partition. There is an intimate association of pedicel and entire bracteole with each of the two lowermost tertiary branches of each inflorescence branch.

E. pulcherrima, Willd.

The origin and the general course of the vascular supply to the inflorescence of this species are in grosser aspects similar to those of *E. clusiae-folia*, but in minute detail some suggestive variations have been observed. Throughout the extent of the vascular supply, the siphonostelic condition seems very apparent. Near the base of the peduncle (Fig. 32) there appears a narrow, unbroken siphonostele with very well-defined radial rows of highly lignified primary xylem (Fig. 32, *x*). At a higher level, the stele becomes dissected by the departure from it, in quincuncial order, of five large fan-shaped groups and three smaller masses of xylem (Fig. 33). The former (Fig. 33, *p*) constitute the five primary steles, each of which is destined to provide the vascular elements for one section of the inflorescence, while the latter three masses (Fig. 33, *q*) unite to form the central siphonostele which supports the pistil (Fig. 35, *q*). Instead of the simultaneous reorganization of each primary stele into four parts, there is in this species a definite sequence for the departure of these four masses from the original primary siphonostele. The leaf trace which supplies the involucral bract arises first (Fig. 35, *b*) and proceeds outward through the cortex in a more or less horizontal course. Before the leaf gap caused by this foliar trace closes, there is a slightly oblique outward passage of a group of xylem elements from each of the edges of the stele adjacent to the gap (Fig. 36, *g*, *g'*). These traces are branch traces, which they resemble in spite of their reduction in origin and structure. In some lateral inflorescences the double nature of the branch trace is clearly evident (Fig. 36, *e*, *e'*), while in other instances both traces originate so closely that they become fused at their very point of origin. This very extraordinary development of branch traces, which pass through the lateral margins of the involucral bracts, may explain in part the apparent absence of the lateral traces of the bract. All leaves of the genus *Euphorbia* possess three leaf traces. The median foliar trace of the involucral bract, however, represents the result of the coalescence of all three original strands, which become distinct at a higher level (Fig. 39, *b²*, *b³*). These branch traces pass

through the involucre to supply the specialized gland. Of the two lateral branches, the right secondary siphonostele (Fig. 36, *R*) departs from its main axis before the left (Fig. 36, *L*), thus continuing the alternate arrangement of the five primary lateral steles. The departure of both branch traces and the leaf trace causes a single gap (Fig. 36, *f*) which soon closes. The continuity of the vascular ring is again established. The xylem within this primary stele (Fig. 37, *i*) reorganizes so as to form two radial rows with a small bundle *p* intercalated between the termini of the rows nearest the pistillate stele. Each of these rows, though not depicting true steles, represent the developing xylem areas in a siphonostele in which there is a strong development of vascular elements on the outer side and an absence of vessels on the inner side of the stele. Eventually each of these rows of xylem divides into eight distinct areas (Fig. 38, *m*), each mass supplying a tertiary branch, which fundamentally is a branch of the preceding relatively main axis, departing from it at a slightly oblique angle. Thus the monochasium becomes established. Coincident with the passing of the vascular strands into their respective branches, the latter separate from their subtending bracteoles and the involucral partitions. In this species the bracteoles do not enclose the entire branch. Parts of the bracteoles adjacent to the tangential planes of each of the branches are obliterated, but next to the radial surfaces they are strongly developed (Figs. 39, 40, *z*). The level at which the vascular strands of the branch enter the filament of the staminate flower is the level at which the remaining portions of the bracteoles divide into definite lobes (Fig. 40, *z*), the distal thirds of which become laciniate. Thus the lateral or radial portion of one bracteole becomes fused basally with the equivalent parts of the bracteole adjacent tangentially. All the lateral lobes of the bracteoles of the branches nearest the involucral commissure basally adhere to the latter (Fig. 39, *c*), but become free near the upper margin of the involucre. All bracteoles arise from the bases of the staminate pedicels.

In the meantime, the bract is traversed for three-fourths of its length by the median bundle. This strand (Figs. 37, 38, *b*) divides radially, giving rise to two lateral foliar traces. By further chorisis each of these two strands multiplies until a single involucral bract is traversed by several strands (Fig. 40, *b*, *b²*, *b³*). Except the lateral branch supply of the second involucral bract, and the adjacent lateral siphonostele of the fifth bract, this reorganization obtains in all vascular areas of each bract of the gamophyllous involucre. The two marginal foliar bundles as a result of their subsequent behaviour become distributed throughout the gland (Fig. 41, *b²*, *b³*), which in this species is solitary. The behaviour of the branch traces is quite noteworthy. By radial chorisis, accompanied by a slight outward migration, each strand multiplies until the tangential band resulting from one secondary branched stele and its adjacent lateral foliar bundle (Fig. 42, *g*, *g'*)
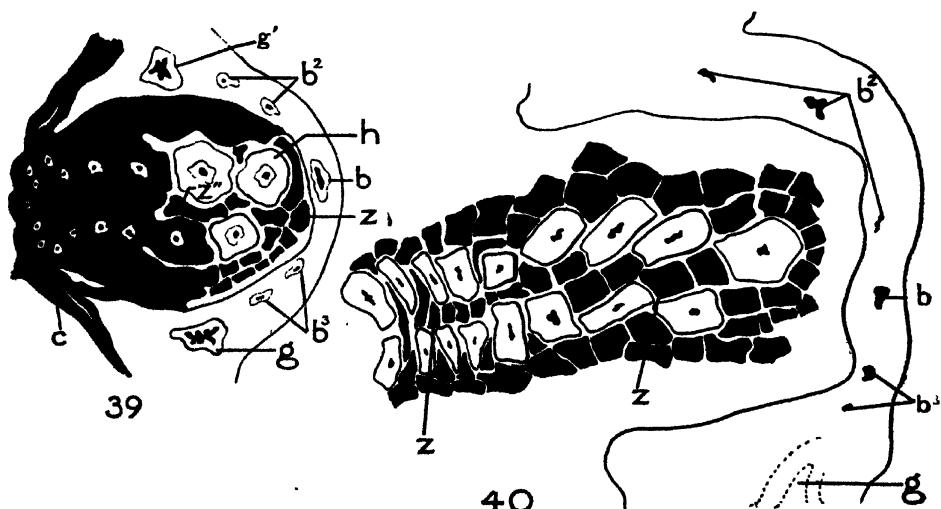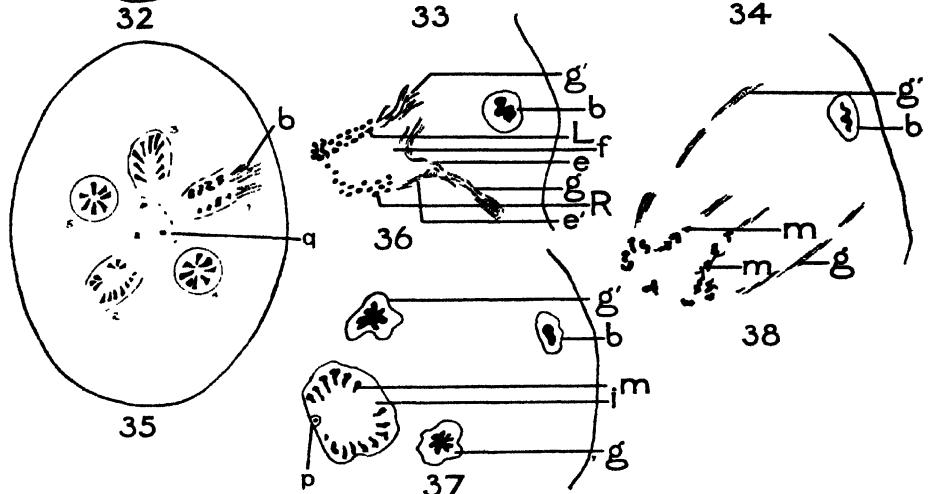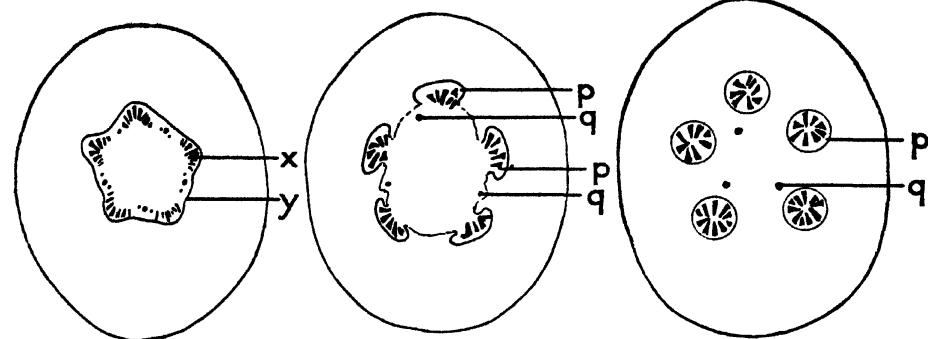
FIGS. 32–40. *E. pulcherrima*. Diagrams illustrating the mode of origin and early behaviour of the vascular supply of the cyathium.

Z z

becomes contiguous with the adjacent tangential band of similar origin; eventually assuming a decided crescent-shaped mass of vascular tissue between inflorescence branches 2 and 5 (Fig. 43, $g + g'$). By a reorganization of the xylem a siphonostele is evolved with its greatest diameter in
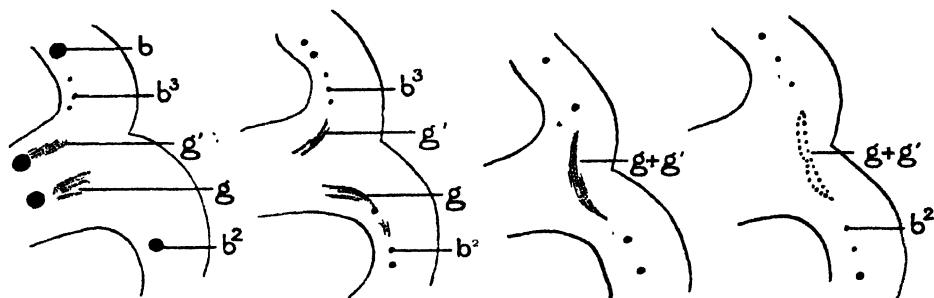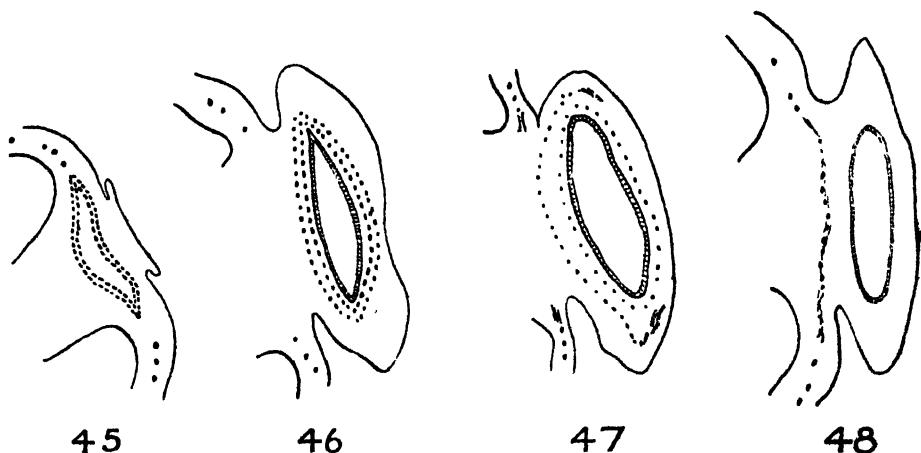
FIGS. 41–8. *E. clusiaefolia*. Diagrams to illustrate the origin and course of the vascular supply of the gland.

a tangential plane (Fig. 44). The ring of vascular tissue which is concentric with the opening of the gland appears in close proximity to the layer of secretory tissue. At the base of the secretory lining the entire cylinder of vascular elements divides tangentially (Fig. 45), forming an outside vascular ring which is concentric with the original cylinder of xylem strands (Fig. 46). Towards the distal margin of the gland there is a fading out of the vascular strands of the outer ring (Fig. 47), and later, portions of the inner ring

disappear among the parenchyma cells of the gland. The petaloid appendages are absent in this species.

Fig. 49 recapitulates on a larger scale the origin of the vascular supply of the gland. All to the left of the median line n represents the supply from one inflorescence branch, and all to the right, from the adjacent inflorescence branch. g, g' represent branch traces which increase their respective
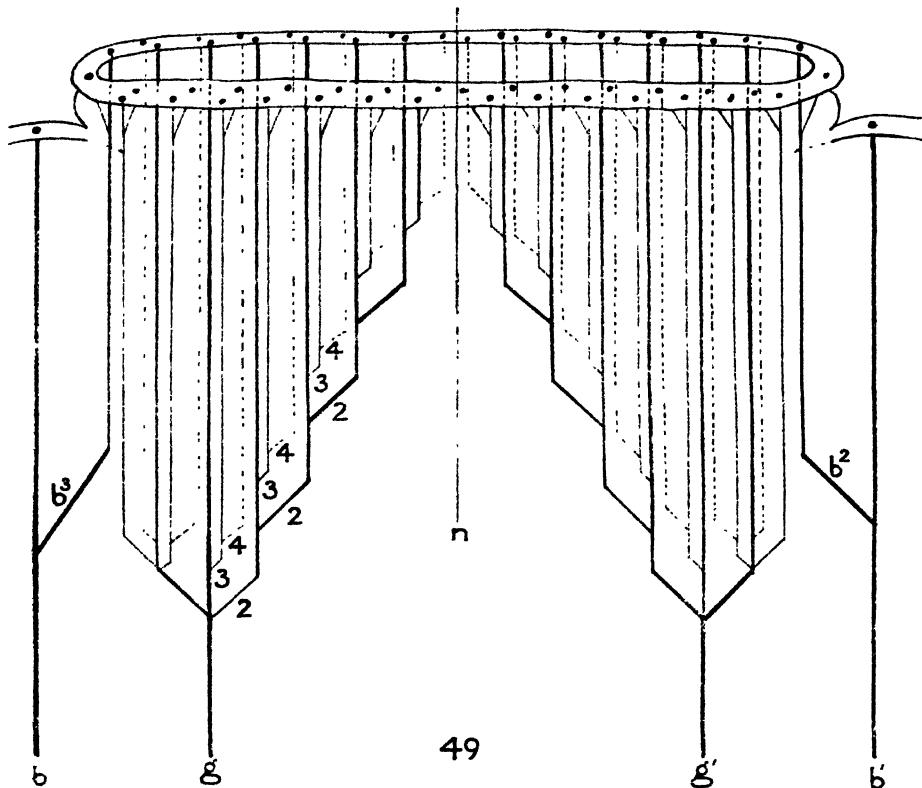
FIG. 49. *E. pulcherrima.* Diagram to illustrate the course of the vascular strands of the gland.

rami first by means of successive radial chorises, which give rise to branches 2. At various levels, each of these by successive tangential divisions forms branches 3 and 4, which upon reorganization form a distinct siphonostele. This stele is increased in its tangential diameter by a similar behaviour of the lateral bundles b^2, b^3 of the involucral bract strand b and b'. By a final tangential division each strand 3 contributes to another ring of tracheae inside the former.

Fig. 50 diagrammatically represents a median longitudinal section of one-half of an inflorescence to show the course of vascular tissue, the rela-

tion of the latter to the monandrous flower *s*, and the morphology of the bracteoles *z*. Dotted lines (except *L*) represent the abortive branches whose absence tend to form a monochasium of tertiary branches. The origin and the distribution of the vascular supply to the gland *g* also is
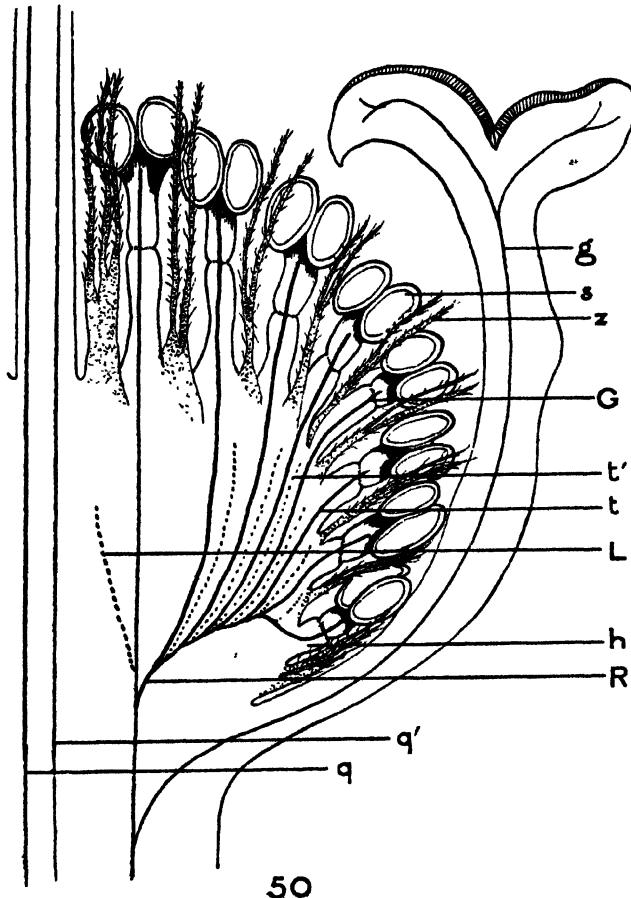
FIG. 50. *E. pulcherrima.* A median longitudinal section of one-half of an inflorescence.

shown. Note that the bases of the bracteoles *z* and their respective branches *h* are fused to form portion of the so-called ' receptacle ' of the ' flower'. *L* represents the left secondary branch of the dichasium, which develops in the same manner as *R*. *q* and *q'* represent two of the three vascular strands in the pedicel of the pistillate flower.

Fig. 51 diagrammatically represents the entire vascular system of the cyathium, exclusive of the pistillate flower. Nos. 1–5 represent the
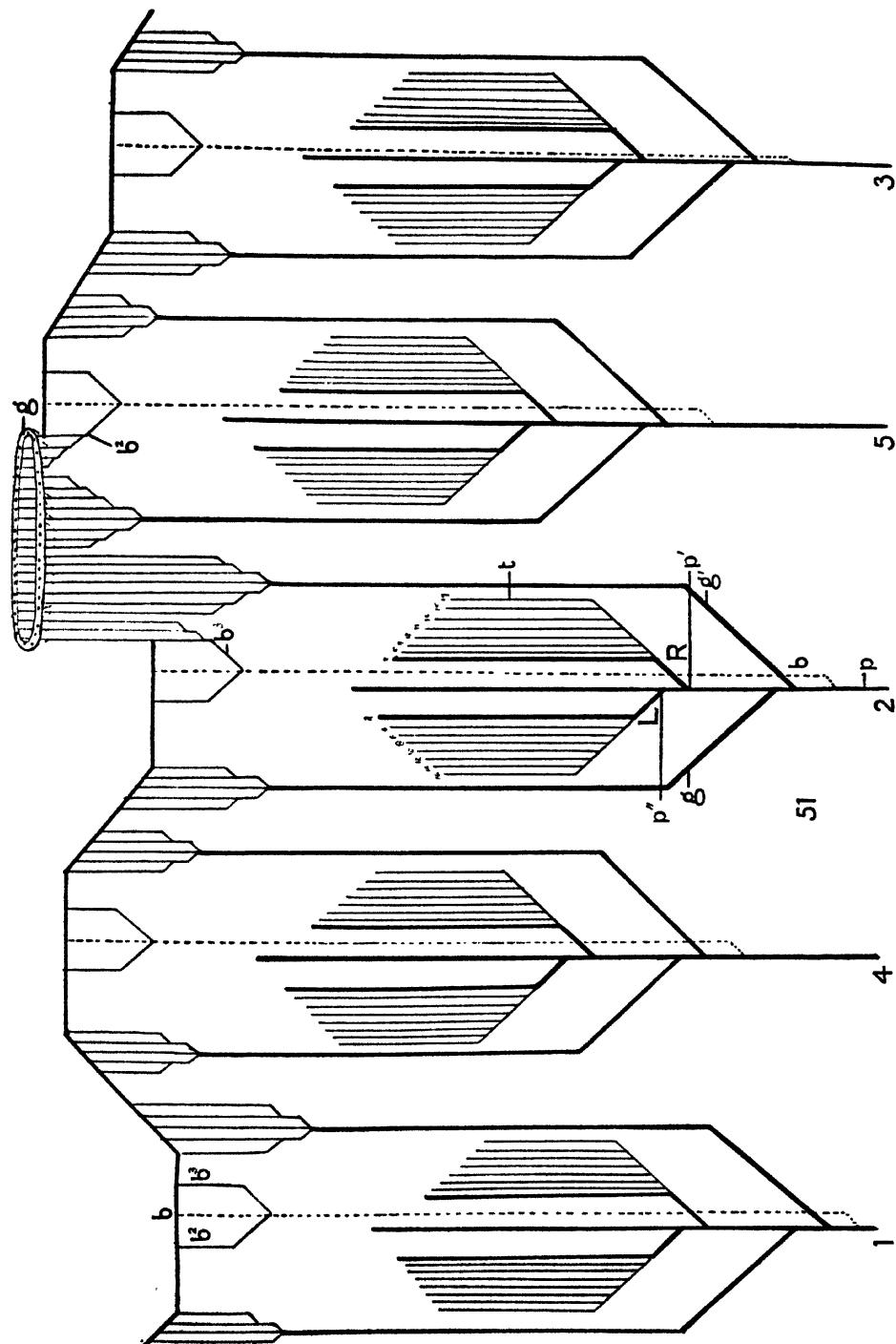
FIG. 51. *E. pulcherrima.* Diagram representing the entire vascular system of the cyathium, exclusive of the pistillate flower.

primary axes, each of which gives off a leaf trace *b* to supply the involucral bract, and at different levels two secondary branches *g*, *g'* which pass through the involucre to supply the single gland *g* situated between lateral inflorescences 2 and 5. A second pair of secondary branches *L*, *R* arises from the main axis *p*. Each of these branches then bears seven tertiary branches (4, 6, 8, 10, 12, 14, 16, or 5, 7, 9, 11, 13, 15, 17), each series of which forms a monochasium. The single foliar bundle *b* forms three foliar bundles *b²*, *b*, *b³*, which supply the bract. In the vicinity of the gland, *b²* and *b³* become incorporated in the glandular supply.

The origin and course of the vascular traces in the pistillate flower present no variation from that in *E. clusiaefolia*. The ovules may or may not develop.

E. pulcherrima embodies primitiveness in its grosser features and specialization in its minor details. The presence of a greater number of monandrous flowers as a result of a greater number of tertiary branches in the monochasium is indicative of primitiveness. This primitive condition is strengthened by the presence of three foliar bundles in the involucral bract. Specialization is evident in the reduction in the number of glands from four to one, in the well-defined stelar nature and complexity of the vascular supply to the gland (which confirms the idea that the gland is at least a modified branch), in the reduction in the size and extent of the bracteoles, and in the disappearance of the vestigial traces representing the inner row of the tertiary branches of the secondary siphonosteles. These traces suggest the probable ancestral status of the tertiary branching.

E. fulgens, Karw.

Except for a few minor variations which may be suggestive, the mode of vascular supply to individual flowers is similar to that in the type. The stele in the base of the ' receptacle ' consists of a regular five-lobed structure (Fig. 52). Intercalated between the bases of adjacent lobes are huge areas of phloem *v* in three of which are embedded groups of primary xylem *q*. These serve as the conducting tissue of the pistil. Each lobe of this vascular complex is comprised of a large irregular mass of heavily lignified vascular elements. By the reorganization of the xylem a distinct siphonostele replaces each of the former chaotic masses (Fig. 53). The distribution of the strands is similar to that of the primitive type, except in the reduction of the number of strands supplying the monochasium which involves but two branches. The position of the branches which have been lost in evolution is suggested by masses of phloem in which the xylem elements have become obliterated (Fig. 55, *v*). At the base of the involucre, the gap left by the departure of the involucral trace (Fig. 54, *b*) divides the remaining primary xylem in halves *L*, *R*, from the outer ends of which are given off two traces (Fig. 55, *g*, *g'*) each conforming to the usual branched

siphonostele of other species. Immediately the remaining vascular elements organize in such a manner as to result in seven definite strands, each entering one of the monandrous flowers.
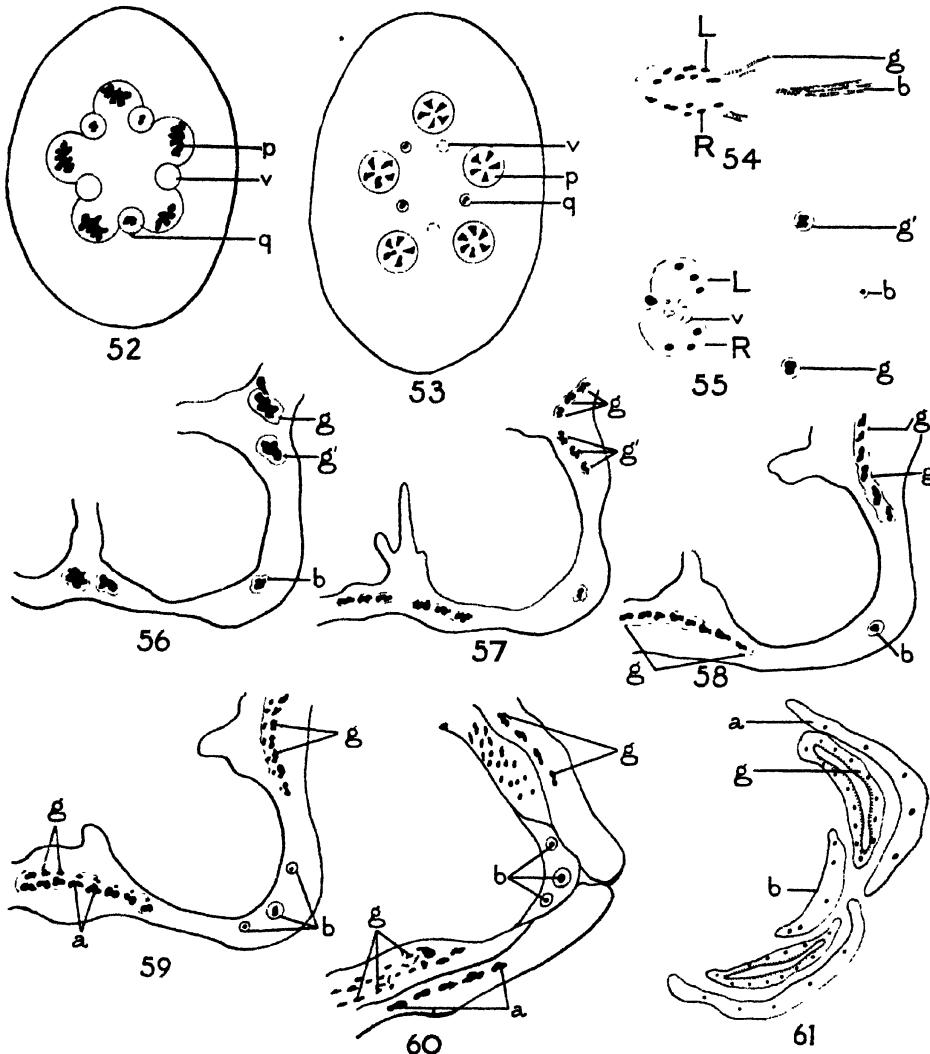
FIGS. 52–61. *E. fulgens.* Diagrams to illustrate the origin and mode of behaviour of the vascular supply to the gland, bract, and appendage.

The scales or bracteoles of the older flowers for a short distance are united to form the involucral partition, but become free and highly fimbriated in their distal extremities. The bracteoles of the three youngest flowers arise from the bases of their respective branches.

When all the stamens are distinct and all bracteoles freed from their respective branches or from other bracteoles, the secondary branch traces (Fig. 57, g, g') by radial division increase in tangential diameter (Fig. 58, g, g'). This continues till the tangentially expanded xylem of one bract meets the similar xylem of the adjacent bract, thus forming a continuous area in a tangential plane (Fig. 59, g). By chorisis, tangential in this case, inwardly, two rows of vascular tissue are formed (Fig. 60, a, g). The outer row a represents the main axis of this branch trace and persists as such and extends into the scarlet petaloid appendage of the cyathium. The inner row, both by repeated tangential and by radial divisions, forms a large mass of scattered vascular groups (Fig. 61, g) which rearrange themselves to produce two distinct rows, eventually surrounding the secretory surface of the gland (Fig. 61). Coincident with this behaviour of the gland traces is the noteworthy activity of the involucral trace, which by radial splitting forms the two original lateral foliar traces (Figs. 59, 60, b). The latter traces divide, forming five bundles in the bract (Fig. 61, b).

Fig. 62 illustrates in a schematic way the vascular distribution of the ·cyathium, exclusive of the pistillate flower. p is the primary axis ; b, the origin of the involucral bract trace ; g, the first secondary branch trace which partially supplies the gland g ; g', the alternate secondary branch trace ; L, the left lateral branch of the dichasium ; R, the right lateral branch of the dichasium ; t, the tertiary branches forming the monochasium ; b^2, b^3, the lateral branches of the leaf trace b ; g, the gland ; a, the petaloid appendage.

Figs. 63 and 64 represent median longitudinal sections through an inflorescence. Fig. 63 is taken through a branch of an inflorescence and its subtending involucral bract. Fig. 64 is a section through the gland and its appendage, primarily to show the origin and behaviour of the branch trace to the gland. The dotted lines represent the median plane of an inflorescence branch which would not appear in this plane of the bundles of the bract. In Fig. 63 the foliar trace b divides radially to form two lateral traces b^2, b^3. In Fig. 64 g is the secondary branch trace which divides at its terminus; the outer branch a continuing into the petaloid appendage and the inner branch g into the gland, in which organ another division is instigated to complete a siphonostele.

In both diagrams R illustrates the primary stele before branching for the monandrous flowers sets in. S represents a single monandrous flower with its anther, and its pedicel separated by the articulation G ; c is the vestige of the bracteole fused basally to the involucral commissure ; z, the entire bracteole surrounding the pedicel.

E. pilulifera, L.

The very small size of the cyathium of this species accounts for the
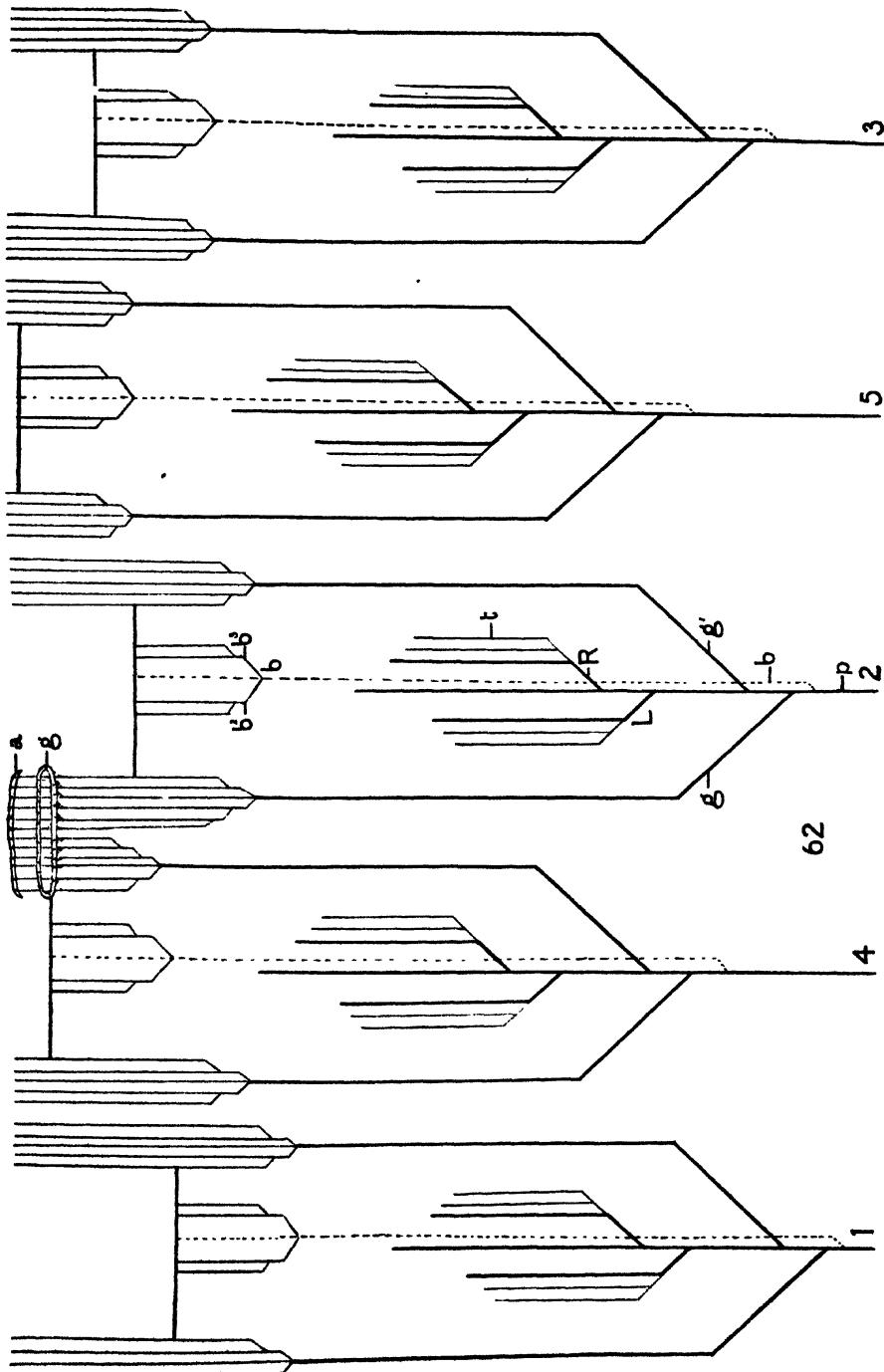
FIG. 62. *E. fulgens.* Diagram representing the entire vascular system of the cyathium, exclusive of the pistillate flower.

extreme reduction of the inflorescence. The various primary steles have lost their identity as such and are represented merely by a few vascular strands forming a central mass. The loss of the typical stelar nature does not deter from the behaviour of these strands as typical steles. This minute primary axis gives rise to the median involucral bract trace, which later separates into its constituent bundles. The lateral foliar bundles, as well as
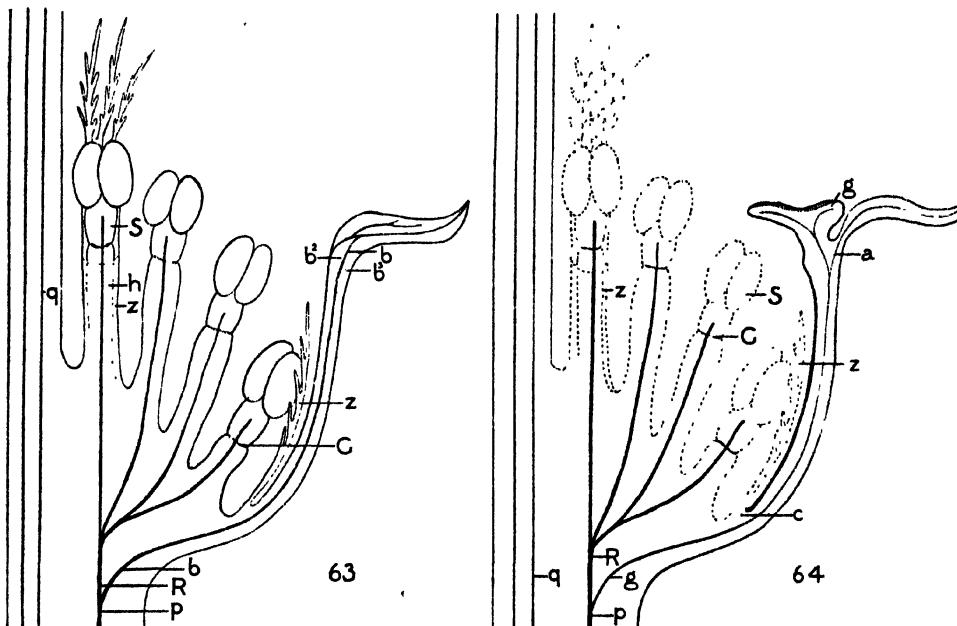
FIGS. 63 and 64. *E. fulgens.* Representations of median longitudinal sections through an inflorescence.

the pair of secondary branch axes which traverse the bract, terminate without anastomosis in the diminutive circular glands. The gland may or may not bear rudimentary petaloid appendages. At this level the primary axis is represented by a small strand of three or four elements which without further reorganization supply the single monandrous flower. Each staminate branch is subtended by a small portion of a bracteole which emanates from the base of the flower but soon becomes fused with the involucre. The distal half of each bracteole is divided into several filaments.

This species represents one in which there has been the greatest amount of reduction in the inflorescence. All tertiary branches of the dichasium have disappeared completely. The primary axis is the only vestige of a highly branched inflorescence. Macroscopically this cyathium may easily be construed as an ordinary flower.

E. splendens, Bojer.

The cyathium of this cactus-like species seems to have buried in its apparent receptacle two abortive branches which heretofore have not been observed in any other species. The peduncle of the inflorescence is supported by an oval ring of strongly lignified vascular tissue surrounded by six equidistant leaf traces (Fig. 65, *A*). By radial chorisis these leaf traces increase to twelve, which gradually move outward through the cortex towards the periphery (Fig. 66, *A*). These latter traces function as the supply to the two broadly ovate scarlet bracts. Coincident with this latter migration, in quincuncial order, is a departure from the main axis of five steles which supply the inflorescence (Fig. 67, *p*). Previous to the segmenting of these steles, each region of parenchymatous tissue between the scarlet bract area and the stele just formed (Fig. 68, *B*, *E*) gives rise to an abortive branch *E* with its two subtending bracts *B*, *B'*. All three rudimentary organs lack any trace of vascular tissue. The scarlet bracts which enclose these extra abortive secondary branches no doubt subtend the latter. The severing of the two outer bracts from the main floral mass is coincident with the rapid proliferation and consequent division of each of the five primary steles into three masses (Fig. 69, *p*). The two outer segments give rise to the second pair (in other species, first pair) of secondary branches (Fig. 70, *g*, *g'*), which are functional in that they become distributed through the glands which are devoid of petaloid appendages. The central stele (Fig. 71, *i*) gives rise to the supply for the remainder of the inflorescence and its subtending involucral bract.

The order of departure of these traces is quite suggestive. The branch traces (Fig. 71, *g*, *g'*) pass out in advance and finally establish themselves as the strands to the glands. This migration is followed by the departure of the single involucral trace from the central stele *b*. In all other species the median involucral trace passes off first and branch traces subsequently, leaving for the primary axis only a few tracheae. Thus instead of the organization of the primary axis into four masses, as in all foregoing species, in this species it divides into three masses, the fourth in other cases being severed from the median stele or primary axis (Fig. 72, *L*, *R*, *p*). After the separation of the involucral trace, the further behaviour of the remaining siphonostele is in accord with the usual custom, resulting in the presence of a reduced inflorescence branch with but five stamens (Fig. 73, *h*). A small outer portion of each bracteole subtending a tertiary branch still persists. The outer segments of each two adjacent inflorescences are fused basally to form the involucral partition (Fig. 73, *c*).

Throughout the behaviour of all the primary steles the main central axis of the entire cyathium does not lose its identity. It consists of a strong vascular siphonostele (Fig. 74). Before the stamens become separated
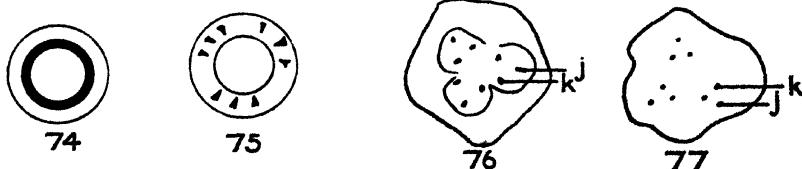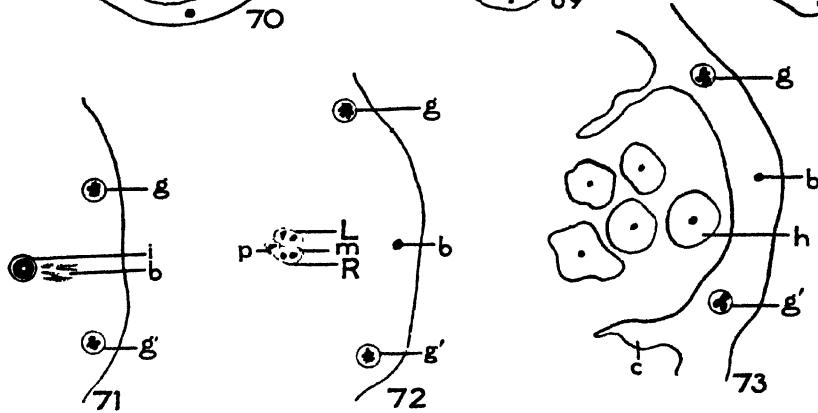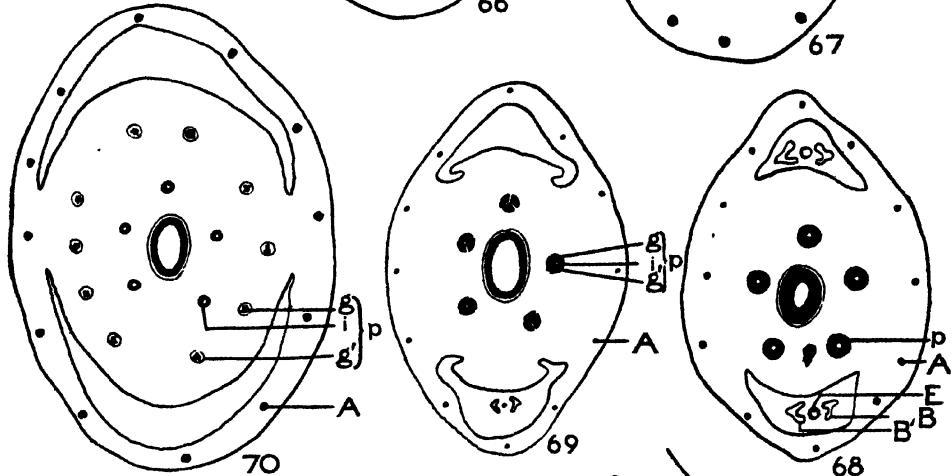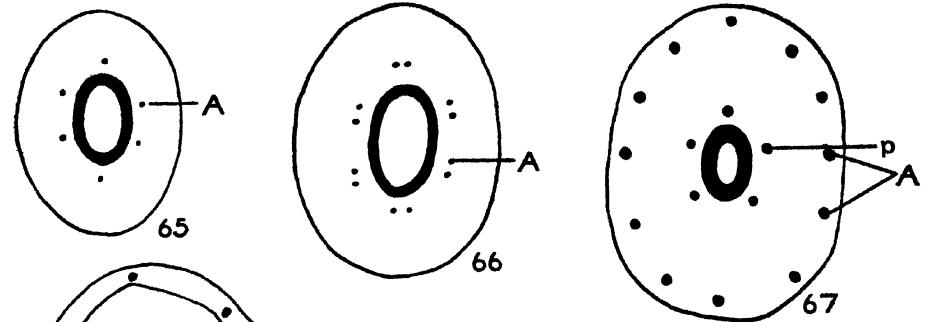
FIGS. 65-77. *E. splendens.* Diagrams to illustrate the origin and behaviour of the vascular supply of the cyathium, including the strands to the pistil.

from the general parenchymatous floral mass the siphonostele reorganizes into nine segments (Fig. 75), three groups of three segments each. Each group represents the usual carpellary supply, a dorsal and two marginal traces (Figs. 76, 77). The subsequent behaviour of these traces conforms to the type in *E. clusiaefolia.*

In grosser aspects *E. portulacoides, E. dentata, E. marginata, E. insularis, E. buxifolia,* and *E. crenulatus* have a close affinity with one of the previously described species. In minor details there is a variation in the number of monandrous flowers, in the presence and absence of petaloid appendages, in the number of foliar traces and glands.
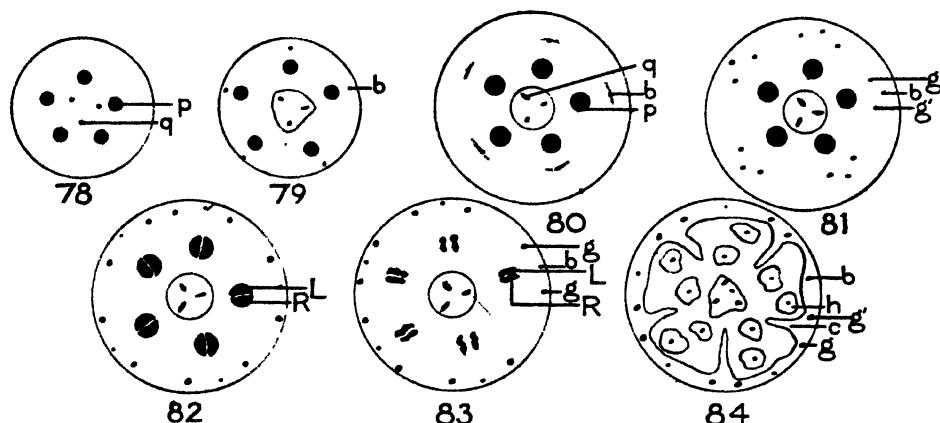
FIGS. 78-84. *E. hirsuta.* Diagrams to illustrate the origin and behaviour of the vascular bundles of the cyathium.

GROUP II.

E. corollata, L.

Great variability and lack of stability in the number of stamens present are marked characters of this species. The variations in the numbers of monandrous flowers from two to four or five within a single inflorescence branch, as well as in and among the various cyathia ; the presence in some peduncles of distinct steles with pith still *in situ* ; the behaviour of these steles in such a manner as to give rise to the foliar trace succeeded by the two lateral branch traces to innervate the glands; and the formation by the bracteoles of a collar about the primary axis of the dichasium—all of these features tend to establish the fact that *E. corollata* is a fairly primitive species. On the other hand the reduction of the stamens to two ; the presence of pithless steles in some forms and their consequent behaviour to quickly form the one trace which subsequently becomes differentiated into the foliar traces of the bract and the traces to the gland ; the persistence of a few vestiges of the bracteoles arising from the bases of the flowers—all

these features lead one to conclude that *E. corollata* belongs to the more specialized group of genus. The presence of five glands, each with its respective appendage, seems to be a constant structure.

Because of this lack of stability in both anatomical and morphological features, it may be justifiable to recognize this species as a transitional form between the more primitive and the more specialized types.

GROUP III.

In the third group, the origin and the course of the vascular supply to the monandrous flower is uniform throughout the various species. The most conspicuous variation from the species represented in Group I is found in the method of supply to the involucral bract and to the gland. *E. hirsuta* will be used as a type to indicate the striking deviations of this group. The reorganization of the tracheae in the dissected siphonostele of the peduncle results in the formation of the usual five primary steles (Fig. 78). These pithless steles, simulating concentric bundles, contribute first to the supply of the bract (Fig. 79, *b*). This strand temporarily establishes itself near the periphery of the stele, where later the gamophyllous involucre will be free from the remainder of the floral tissue. In this position there is a gradual radial chorisis of this tracheal mass into right and left segments (Fig. 80, *b*). These segments gradually move laterally (Fig. 81, *g, g′*) and slightly upward until they reach approximately the region of the lateral margins of the bracts. Finally, by division, the lateral branches of each of two adjacent inflorescence branches meet and after further tangential chorisis penetrate the simple glands. Thus in this group the involucral bract trace *b* doubtless is the result of a basal coalescence of the two former branch traces *g, g′* and the single foliar trace to the bract *b*, each of which eventually establishes its own identity. Except in *E. petaloidea* the single foliar bundle soon divides into its three common leaf traces. These two lateral foliar bundles also serve as part of the glandular supply. During the outward migration of this first complex strand the remainder of each stele proliferates rapidly, reorganizes, and divides in two (Fig. 82, *L, R*) in order to traverse each of the lateral branches of the dichasium and their respective monandrous flowers (Figs. 83, 84).

The remaining species of this group differ only one from another in the number of glands, in the presence or absence of petaloid appendages and the number of monandrous flowers.

THE PISTILLATE FLOWER.

The pistillate flower which terminates the main axis of the cyathium presents no unusual features. With the exception of *E. splendens* the vascular distribution in each species examined conforms strictly to that

of *E. clusiaefolia.* In a few cases (*E. Palmeri, E. splendens, E. Cyparissias, E. stricta, E. Esula, E. commutata, E. crenulatus*) the siphonostele of the pedicel is very apparent. This stele later divides into three distinct segments, each of which, by further division, gives rise to the dorsal and the marginal carpellary traces to a carpel. In the remaining species examined the three masses are separate and distinct at the base of the pedicel, and so continue for the entire length of the pedicel. About at the level at which the stamens and scales become distinct the marginal and dorsal traces become clearly separated.

DISCUSSION.

The foregoing morphological and anatomical descriptions of the species studied give evidence which seems to reveal the status of the *Euphorbia* flower. That there is, at least among the species investigated, an order of succession which exemplifies evolutionary tendencies within the genus seems to be quite apparent. Relative to the cyathium in general, *E. clusiaefolia* and *E. pulcherrima* represent primitiveness ; while the other extreme, great reduction, is consummated in *E. hirsuta* and *E. Esula.* The gap between these species is bridged by a very representative series transitional along various lines. Reduction accompanied by specialization is well portrayed among the members of Group I and Group III, with *E. corollata* as a transitional species. The same principle is exhibited in the glands of *E. dentata* and *E. pulcherrima.* The decreasing number of monandrous flowers verifies the theory of reduction. The organ the morphological nature of which has been least understood is the bracteole, subtending the individual monandrous flower. In one species it seems to be present in its entirety ; in several it is merely vestigial, while in a small group it has disappeared.

The Inflorescence.

Among the investigators of the ' flower ' of *Euphorbia* three views have been produced. Tournefort (1700) (67), Linnaeus (1753) (45), Payer (1857) (53), and Baillon (1858) (2) concluded that the true status was that of a single hermaphroditic flower. Lamarck (1786) (42) considered the cyathium an assemblage of smaller flowers. The bracts, according to his view, form a common calyx ; the bracteoles between the groups, the calyx proper of each staminate flower. The most generally accepted view of the present day was first suggested by R. Brown (1818) (18)—that within the involucre there is a complicated androgynous inflorescence in which the pistillate flower is the ovary, and the monandrous flowers are reduced stamens, forming lateral inflorescences arising in the axis of peripheral leaves which serve

as bracts.   Adr. de Jussieu (1824) (39), Roeper (1824) (59), Wydler (1845) (76), Alex. Braun (1853) (15), de Candolle (1866) (23), Warming (1873) (70), Bentham and Hooker (1883) (7), Eichler (1875) (25), Worsdell (1903) (74), and others accepted the basic principles of Brown's theory, disregarding some minor details.

The vascular anatomy reveals features which substantiate these conclusions of so many students, that the cyathium is an inflorescence and not an hermaphroditic flower.   The behaviour of the stele of all subsequent branches, and of the pedicels of the monandrous flowers, is definite proof that this is an inflorescence.   The vascular system of the peduncle of the inflorescence is divided into two general types as suggested by Groups I and III respectively.   In the majority of the species of Group I it forms a normal siphonostele.   In a few species the vascular cylinder becomes a dissected siphonostele.   Because of reduction, in many branches of the inflorescence, the siphonostele has contracted to an irregular mass of xylem, and the pith has vanished.   In a normal flower the stele of the peduncle gives rise to various traces which supply the lateral appendages of the axis; namely, the sepals, the petals, stamens, and carpels.   This is not the case here.

In each group the main axis of the inflorescence is supported by the siphonostele, but gives rise to six steles, and not to six traces.   The five outer steles are designated the lateral primary steles; and the sixth, or central, the pistillate stele.   Each of these lateral primary steles is a branch trace arising from the main axis by means of the two usual strands. *E. pulcherrima*, in some of its groups within the cyathium, furnishes substantial proof of this statement (Fig. 36, *e, e'*).   In the majority of cases the great suppression of floral parts has brought the origin of the branch traces in such close proximity one to the other that their separate identity is entirely lost.   Consequently the stelar nature of these primary axes is evident a few microns from the point of origin.

The main axis of the entire inflorescence does not give rise simultaneously to the constituent branches, but shows a spiral arrangement effecting a two-fifths phyllotaxy (Fig. 9).   By the reduction of the floral axis, and the suppression of internodes between branches, this quincuncial sequence, which is an important phyletic character, has become obscure, simulating a whorled condition.   However, each primary axis moves outward in the cortex, and as it does so gives rise to its subsequent branches and respective bracts in definite sequence.   Beyond this point the spiral sequence is lost, due to extreme suppression (Figs. 10, 11).

The subsequent behaviour of each primary stele is added proof of the presence of an inflorescence.   In *E. clusiaefolia* the reorganization of this primary stele eventually gives rise to four vascular masses, three of which are decidedly stelar in nature and behaviour, and the fourth, a normal

foliar trace. The two lateral tracheal cylinders of this triple group initiate the greater part of the glandular supply. If glands are absent, these strands, as customarily, ramify through the involucral bracts, in the upper margins of which they terminate.

The second group of species, all of which possess contracted steles, show a peculiar behaviour of the primary axis. From each of the lateral primary axes is severed a large segment which gradually moves to the stem periphery. By radial chorisis the tracheal mass initiates a right and left segment. The median segment is equivalent to the foliar trace of Group I (Fig. 89, *b*). The lateral segments, by moving in a tangential plane till they approximate the involucral commissure, form the branch
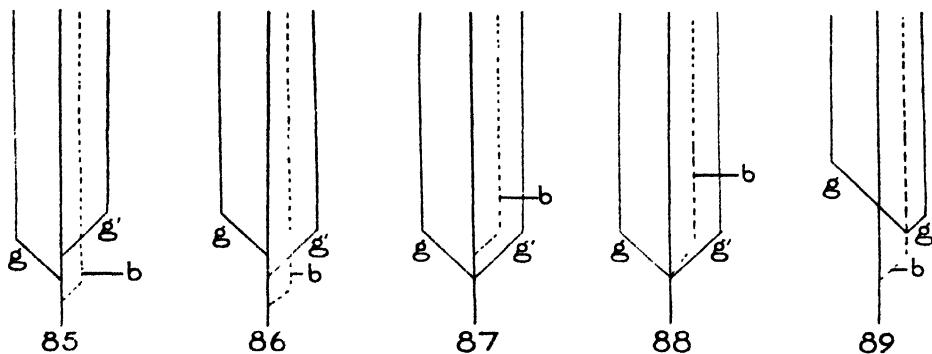
FIGS. 85-9. Diagrams to show the various types of origin of the bract and gland traces. 85. *E. fulgens.* 86. *E. pulcherrima.* 87. *E. splendens.* 88. *E. lusiaefolia.* 89. Group III.

traces *g*, *g'*. Figs. 85-9 will serve to recapitulate the origin of the first pair of secondary branches and the foliar traces, and their relation one to another.

Group I shows much diversity in the origin of these first vascular traces. The second group, comprising the majority of species, is more constant in its behaviour. However, there seems to be a well-established series in the first group—alternate (Figs. 85, 86), opposite (Fig. 87), and whorled (Fig. 88) divergences. Phylogenetically the alternate phyllotaxy of axillary branches in cymose inflorescences is the most primitive type, while the opposite system is a derived one, due to the suppression of internodes. Hence in the apparent opposite condition of the floral members, one of any pair of branches will become the lower and the first of the two to develop. Where suppression has become well established, a whorled condition may become quite apparent. Therefore the opposite status is not primitive, but secondarily derived from the alternate.

In the second group, the suppression has reached such a state as to cause a coalescence of the first three traces of each primary axis for at

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least part of their course (Fig. 89). Eventually they separate into their constituent members—axillary branches *g*, *g'* and foliar trace *b*. The order of departure of these traces of the inflorescence is closely correlated with the arrangement of the vegetative leaves. Therefore the arrangement of the monandrous flowers and leaves is similar, dichotomous or dichasial.

The primary axes from which these branches originate do not lose their identity, but close up and proceed as in normal behaviour. At a higher level in the base of the cyathium, these axes, which are represented in all groups by a small trace, terminate in a monandrous flower, thus forming a determinate inflorescence. By some authors this first and oldest flower of each group within the cyathium has been designated as the primary staminate flower. In the majority of species the primary axis, just previous to its terminus, gives rise to a second pair of secondary branches, thus completing a dichasium (Fig. 12, *L*, *p*, *R*). Thus each primary axis which is borne in the axil of an involucral bract situated on this axis is, in its first stages, a very simple cymose inflorescence, consisting of three flowers—a central and two laterals which terminate secondary axes (Fig. 97). In *E. lucida*, *E. commutata*, *E. petaloidea*. *E. Darlingtonii*, *E. Helioscopia*, and *E. calcicola* this represents the extent of the inflorescence within a single group. In *E. arkansana*, *E. Cyparissias*, *E. hirsuta*, *E. albomarginata*, *E. buxifolia*, and *E. crenulata* the primary axis is lost at the node from which the two branches of the dichasium originate (Figs. 96, 98). In *E. Esula* extreme reduction has resulted in the retention of but a single branch (Fig. 99). Since the primary steles are such that they result in only a small trace as the continuation of the primary axis, and such huge steles for the dichasial branches, it seems natural to infer that where but a single branch is retained in each inflorescence, apparently it must represent one of the laterals and not the main axis.

The dichasial method is fundamentally the method of branching in the *Euphorbia* inflorescence. Many species possess more than three floral branches. This is brought about by the formation of tertiary branches from the dichasial branches. Among the various species the number of rami vary, ranging from one (Fig. 94) to seven (Fig. 90). The resulting type is a monochasium. This final inflorescence type is not a primitive one, or one first in evolution, but a system which has arisen by reduction of the inner lateral branches of what would have been a simple dichasial branching. Since the lower of the two lateral branches in a simple dichasium arising from alternate leaves develops first, it is this lateral ramus which matures, and the upper or the one turned towards the main axis which aborts.

Definite proof to corroborate these last statements is given in *E. clusiaefolia*, where, in the base of the cyathium, the steles in their

reorganization to supply the monandrous flowers gave rise to several small strands of tracheae towards the inner peripheries of two adjacent steles (Fig. 13, *v*). These masses soon become obliterated in the parenchyma of the stele. In *E. marginata* the abortive branches are represented by small areas of non-lignified tracheae ; in *E. fulgens* these tertiary branches are mere vestiges, represented by phloem, as suggestive areas (Fig. 55, *v*). In some cyathia of *E. clusiaefolia* a few inflorescence branches revert to ancestral conditions, in that two inflorescence branches developed normal tertiary branches, thus forming a continuous dichasium rather than a monochasium. In another group one entire branch of the primary dichasium developed completely, while the other branch developed but the monochasium, the normal status. In all other species examined no traces or vestiges of these abortive tertiary branches were discernible. The first functional tertiary branches originate from the right side of the right lateral branch, and from the left side of the left branch of the dichasium (Fig. 15: 4 and 5). The apparent zigzag arrangement is caused by the alternation of the origin of one branch of one monochasium with a similar branch of the opposite monochasium of the same group.

Thus the presence and the behaviour of the steles gives conclusive proof that the cyathium is not an hermaphroditic flower, but a complex inflorescence. The presence of the branch and foliar traces ; the formation of two systems of ramification, dichasium and monochasium; the vestiges of tertiary branches, and the establishment of a definite system of phyllotaxy, point to this cyathium as a highly specialized inflorescence, due to suppression of nodes and internodes, aggregation of branches, the abortion of branches, and the unequal development of lateral rami of the dichasium.

The Involucral Bracts.

The diversity of opinions regarding the nature of the inflorescence has brought forth equally as many interpretations regarding the nature of the gamophyllous involucre. Linnaeus (1753) (45) believed the involucral bracts were petals ; Payer (1857) (53) and Baillon (1858) (2) considered these structures as sepals ; while the majority of the later students have interpreted these segments as bracts, in the axils of which arise inflorescence branches.

In the previous discussion on the anatomy of the inflorescence it was stated that in all but one species investigated the involucral bract is a phyllome in the axil of which is developed an inflorescence branch ; five inflorescence branches and their respective bracts and glands constituting the cyathium. In *E. splendens* the bract subtends the inflorescence only and not the gland, as the vascular supply for the latter organ originates from the stele before there is any suggestion of the departure of the foliar

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trace.   Morphologically the bracts have been described as forming a gamo-phyllous involucre.   Closer observation seems to favour the idea that the individual bracts are separated by flattened steles which eventually branch and rebranch in order to ramify through the gland, and also through its appendage when the latter is present.   By the congestion of innumerable flowers to form a small floral mass, the lateral margins of the bracts, including some vascular strands, have coalesced with the cortical regions of the steles, the two producing the gamophyllous involucre.   The detailed anatomy of the gland will be discussed later.   The proof that in most cases the bract initiates the breaking up of the primary stele, and that all other branches to the inflorescence arise from the same axis, makes it clear that the bracts, first, must be considered a phyllome, in the axil of which the main inflorescence branches have their incipiency ; and second, that this organ is traversed by a single leaf trace which represents the union of the three normal traces which may become separate in their distal courses.

The Position of the Monandrous Flowers.

In the discussion of the inflorescence it was stated that, except in a few species, the axes of the monandrous flowers originated after the passing out of the branch and foliar traces.   The resulting medullated vascular cylinder divides into three masses ; two large steles and a smaller one interpolated between the adjacent peripheries of the two steles (Fig. 12).   The smaller one represents the continued primary axis (Fig. 12, p), and at its terminus gives rise to a monandrous flower which has been designated as the primary staminate flower.   This primary axis (Fig. 12, p) gives rise to a pair of secondary branches (Fig. 12, L, R), thus completing a dichasium.   Each lateral branch may depart from the stele at the same level, but in the majority of species one branch arises slightly above the other.   Thus in *E. lucida*, *E. commutata*, *E. petaloidea*, *E. Darlingtonii*, *E. Helioscopia*, and *E. calcicola* the arrangement of the vascular tissue is quite alternate, and the oblique passing out of the main axis first of one branch and then of the other is quite evident.   Even though the subsequent branches may be equidistant from each other, within their respective monochasia, the fact that the first branches do not rise simultaneously would indicate an irregularity between the two rows (Fig. 15).   There is a definite sequence in the order of origin of each successive branch from the preceding one. After the formation of branches of the dichasium, the next branch formed is the one from the lowermost ramus of the dichasium and may be designated as branch 4, thus 4 originating from 2 (Fig. 15).   The next in order is the one from the second branch of the dichasium, the third branch, which gives rise to the fifth.   The vascular elements of each branch arise towards the outer margin of each monochasium, by a slightly oblique

chorisis of the preceding axis. Thus the vascular supply of the individual monochasium does not arise in a perfect straight line, but in a curve towards the median axis of the primary inflorescence branch. The reduction in the number of monandrous flowers lessens the curvature resulting from the oblique origin of each successive ramus. The suppression of floral parts, no doubt, may explain this order of development which gives rise to a scorpioid cyme, a monochasium. As to the type of monochasium it is difficult to conjecture, for extreme suppression in the base of the cyathium causes a very rapid proliferation of the vascular strands to form consecutive branches. However, it may be stated with some degree of accuracy that in the most species examined the successive vascular strands to supply the pedicels of the monandrous flowers arise from the same side of the main axis.

To recapitulate (Fig. 15), it may be stated that the first monandrous flower arises on the terminus of the main axis; the second arises just below the terminus, and with the third, slightly above the second, simulates a dichasium. Where more than three flowers are present each branch arises from a preceding relatively main axis, that is the fourth from the second, the fifth from the third, the sixth from the fourth, and the seventh from the fifth, &c. As a result the monochasium, the lateral axis of which always forms on the same side of the relatively main axis, is established.

The Monandrous Flowers.

Literature reveals the fact that there is more nearly a consensus of opinion regarding the development and the schema for the arrangement of the monandrous flowers, but less consistency in the interpretations of these organs.

To Adanson (1763) (1), Payer (1857) (53), Baillon (1858) (2), and Pedersen (1873) (54), these fundamental structures were articulated stamens. Schmitz (1871) (63) believed that the anther was a metamorphosed axis, while Čelakovsky (1872) (21) and Strasburger (1872) (66) concluded that the flowers were composed of two sessile stamens. Schmidt (1906) (62) found good analogies in the staminate flowers of several isolated genera of the same family. In these genera the 'perigon' was found above the articulation; and accordingly he believed that the axillary portion of the stele terminated with the constriction and the upper half was phyllome in nature.

This is but a meagre survey of the various conceptions. Anatomical evidence of the nature of the pedicel follows. Typically the vascular tissue of the pedicel is in the form of a concentric bundle. However, in *E. corollata* a few pedicels were observed in which the vascular supply is a stele with a central core of pith. In other pedicels of the same cyathium, because of great reduction, the pith is lost. In these cases the vascular cylinder is

represented not by a stele, or even by a symmetrical bundle, but by an irregular mass of xylem. The effect of reduction upon the steles is commonly a contraction and loss of pith, so that the remnant of the stele appears as a bundle. This is the condition in aquatic plants and in some other delicate herbs. The part of the pedicel below the articulation is clearly, on anatomical evidence, of axial nature. The region of the articulation then is the receptacle upon which the flower is situated, and in function is similar to that of other receptacles. From this receptacle originates a single foliar trace composed of a few elements which support the stamens (Fig. 18, *s*). Like most stamens it is supplied with but a single trace. The steles in the pedicels are proportionately larger than the traces in the stamens. There is not the slightest evidence that this stamen represents two fused stamens or is the remnant of a group of three.

In ontogeny the vascular strands appear in the pedicel first and later in the filament, as is the normal condition for axil and stamen. Hence the stamen is situated upon a definite receptacle which terminates the axis, a caulome structure, therefore a branch of the monochasium. Other evidence for the presence of two separated structures is the difference in colour between the pedicel and its phyllome. In *E. splendens* the pedicel is a dark purple, the filament a bright red. In *E. portulacoides* the presence of ridges upon all the pedicels of the monandrous flowers and their absence from the filament of the anther is a noteworthy evidence.

In the various species there is quite a diversity in the number of monandrous flowers in an inflorescence branch. For a single species there is quite a uniformity of numbers within one group of a cyathium.

Since *E. pulcherrima* contains seventeen flowers on an inflorescence branch, and *E. Esula* but a single one, with species possessing all gradations between these extremes, this wide variation indicates an evolution from a more or less primitive condition, as shown by the numerous stamens in *E. pulcherrima* (Fig. 90), to extreme reduction, as exhibited by *E. Esula* (Fig. 99). The transition between these two limits shows the line of evolution (Figs. 91-8). This reduction no doubt proceeded in two directions: one in which the primary flower as well as the two laterals were retained, as in the case where three monandrous flowers were present (Fig. 97); the other, in which the primary axis was lost and lateral branches retained as in *E. buxifolia* (Fig. 96).

The articulation of the filament to the pedicel is a constant character of the monandrous flowers of the species observed. It is a morphological feature, the real nature of which has not been ascertained. This articulation may not be apparent at first sight, but macroscopic observations reveal this constriction between the caulome and the phyllome, in other words the pedicel and the filament. The majority of the workers in ontogeny considered this a mere modification of tissue, belated in its appearance. How-

ever, in many closely related genera, particularly in *Anthostoma*, this region bears a whorl of leaflets. Schmitz in *E. Cyparissias* 'found similar conditions which he included in his teratological studies. Čelakovsky also from analogy with other genera considered the articulation a node where leaflets may grow or be suppressed.

Since there is no distinct anatomical evidence of vascular tissue supplying appendages in this area, again we have evidence of the position of an organ which together with its vascular supply has vanished. It seems that the articulation is the position of an abortive perianth.
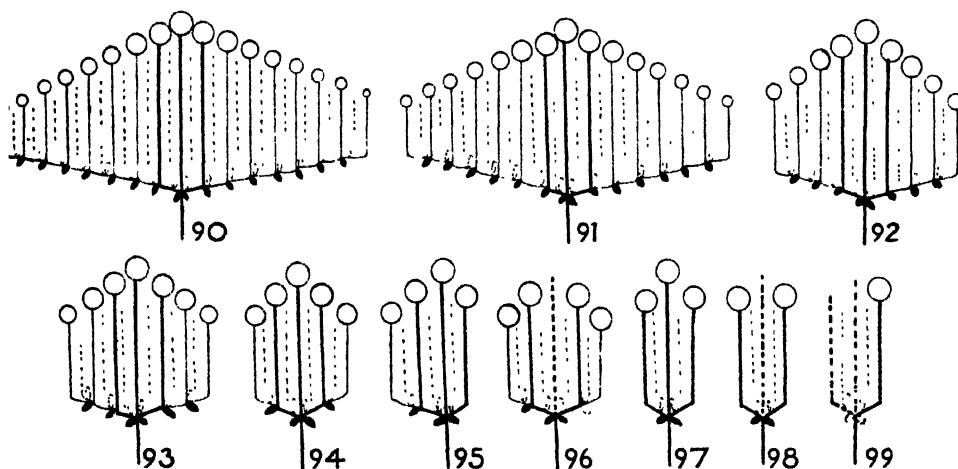
Figs. 90-9. Diagrams to illustrate the vascular supply of an entire inflorescence branch and to show the reduction of the inflorescence. 90. *E. pulcherrima.* 91. *E. marginata.* 92. *E. cordifolia.* 93. *E. portulacoides.* 94. *E. fulgens.* 95. *E. Preslii.* 96. *E. buxifolia.* 97. *E. Helioscopia.* 98. *E. hirsuta.* 99. *E. Esula.*

The fact that the entire branch, caulome with its stamen, is subtended fundamentally by a scale is further evidence that the entire flower has been reduced to a single stamen.

The Bracteoles and their Relation to the Staminate Flowers.

There has been much speculation in the interpretation of these organs (as their presence has been indicated by various investigators). Ontogenetic evidence regarding the morphology of the bracteoles is rather rare, due, as one investigator has stated, to the difficulty in observing their development.

Adanson (1763)(1) considered these nondescript structures as petals of individual flowers. Adr. de Jussieu (1824) (39) and R. Brown (1818) (18) designated them as bracts. Payer (1857) (53) has characterized them as disc structures on the floor of the cyathium. Roeper (1824) (59) and

Wydler (1845) (76) considered the scales as bracts to the staminate flowers. Hieronymus (1872) (36) presented the unique interpretation of interpolated stipules. Warming (1870) (69) considers these structures to be trichomes analogous with the pappus of the Compositae, the spines of the Cacti, the perigynia of the Cyperaceae, the hairs in the inflorescence of *Typha*. Schmidt (1906) (62) in his treatise considers them as bracteoles of rather irregular origin.

Transverse and longitudinal sections through the base of the cyathium of the various species present, relative to the scale, the diversity of conditions, all of which may be arranged in a progression exhibiting an evolutionary series.

From grosser aspects it is suggested that there are two methods of origin of these scales or bracteoles of the monandrous flowers. In many species there is no doubt that they arise from the base of the pedicel (Fig. 39, *s*), the terminus of which bears a male flower. In other species they seem to arise from the involucral partition or commissure, which appears as an inward projection of the involucre between two adjacent inflorescence branches (Fig. 39, *c*). This partition, however, represents the fused bracteoles of the monochasia of adjacent inflorescence branches and not the fused lateral margins of adjacent involucral bracts. These bracteoles become freed one from the other, inaugurating a gradual disappearance of the involucral commissure (Fig. 40, *s*). In some other species the scales subtend the base of the flowers and apparently form the involucral partitions as well.

These scales are composed of a homogeneous parenchymatous cell mass devoid of vascular elements of any type. Here, no doubt, we have structures in which the vascular supply has disappeared; the organ is retained. For quite a distance these bracteoles are fused with their respective branches which they subtend. Nearer the base of the cyathium, it is difficult to differentiate between bracteole and pedicel. These nondescript organs assume various sizes and shapes and become fimbriated or laciniated at their distal ends. In *E. clusiaefolia* (Figs. 100-2) the bases of the bracteoles are fused one to another and surround the pedicel. This situation may have been brought about in one of two ways: first, the scale may have·been of the perfoliate type primitively; or second, the bracteole, because of the crowding due to the compression of the inflorescence, may have spread about the pedicel until it completely encircled it, for a lateral expansion was impossible. Since the individual pedicels are approximated, and the bracteoles subtend these pedicels, these scales surrounding the pedicels naturally cohere and finally become united one to the other, between the individual members of the same monochasium, and between the two monochasia of a single primary inflorescence. For similar reasons the bracteoles on the outer side of each group have coalesced with the

outer portions of the bracteoles of the adjacent group to form what commonly has been called the involucral partition or commissure (Fig. 103, *c*). At various levels in descending basipetal succession, these bracteoles become freed from their respective pedicels and from one another between the groups, leaving no partition or commissure as a distinct involucral partition. Another point in proof of the absence of a true involucral partition formed by the union of the involucral bracts is the fact that these partitions are lost entirely at a level at which all anthers are
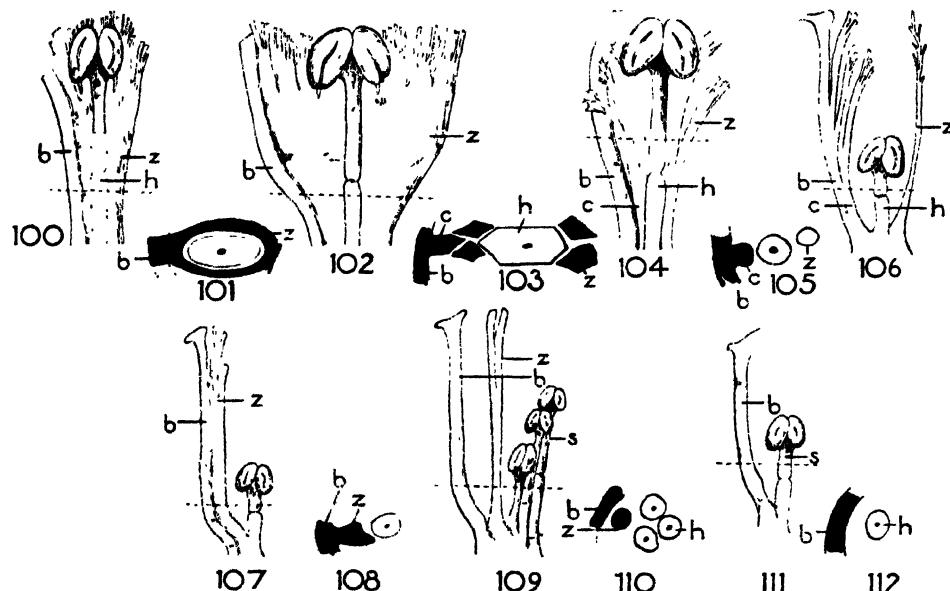
FIGS. 100-12. Transverse and longitudinal diagrams to illustrate the evolution of the bracteole. 100-2. *E. clusiaefolia.* 103 and 104. *E. pulcherrima.* 105 and 106. *E. Darlingtoni.* 107 and 108. *E. portulacoides.* 109 and 110. *E. buxifolia.* 111 and 112. *E. Esula.*

developed. If this is an involucral partition it should be present throughout the entire height of the involucre.

In *E. pulcherrima* (Figs. 103-4) scales do not surround the pedicels of each flower as in *E. clusiaefolia*, but seem to be limited to positions lateral to the pedicels between the monochasia of two adjacent groups, *c*, and between the two monochasia of a single group *z*. The portions of the bracteoles between the individual pedicels of the monochasium have become lost, caused by the apparent shortening of the axis.

In *E. marginata* and *E. dioscoreoides* the entire bracteole is evident in the first-formed and older flowers only, while only vestiges subtend the remainder of the flowers. The remnants are limited to the inner margins of the two scorpioid cymes of the same inflorescence branch, and to the

thickenings between inflorescence branches which are composed entirely of scales.

The majority of species present the condition in which all traces of bracteoles between individual members and between monochasia of the same group have disappeared and only those between monochasia of two adjacent inflorescence branches have been retained. Abortion is carried still farther when all vestiges are lost except a single remnant for each monandrous flower, as in *E. petaloidea* and *E. Darlingtonii* (Fig. 106, *s*). Greater simplicity is reached where the great reduction of monandrous flowers of a primary inflorescence is accompanied by only one small vestige, as in *E. buxifolia* (Figs. 109, 110, *s*). The climax is reached in *E. Esula* (Figs. 111, 112), in which species all vestiges of the bracteole have disappeared and one flower represents a single inflorescence branch.

The species examined bring forth evidence that the bracteole may be represented in its entirety, or may be completely absent. These two extremes are bridged by many transitions. The general condition is that about one-third the length of the involucral cup is united with the axis by means of scales. Thus the scale is met with in every stage of degeneracy, until it has completely vanished and even the vascular cord belonging to it is entirely lost.

Morphological evidence obtained from many species suggests that all scales arise from the base of the pedicel of the monandrous flowers which they subtend or surround and by which they may be surrounded for various distances. Due to the aggregation of branches and to the suppression of nodes and internodes, many of the scales have become united one with the other, forming a partition between the inflorescence branches. This partition is attached to the' base of the cyathium, the scales of which in basipetal succession gradually become separated into their constituents, which represent remnants of the subtending bracteoles. All scales are separated from the rest of the floral group at a level below that at which anthers appear. Up to that level they are fused with their pedicels.

The Glands.

The glands are structures which have received little attention from the investigators' standpoint. A few authors have ventured to give some opinion regarding their interpretations. Linnaeus (1753) (45) and Adanson (1763) (1) considered them sepals; a few others declared that they must be petals. To Baillon (1858) (2) the latter idea was more plausible, in that these structures alternate with the calyx lobes, and often simulate a corolla in form and coloration. The students of ontogeny have abandoned this idea on the ground that these structures manifest them-

selves in the angle between adjacent sepals after the latter, the staminal groups, and a portion of the ovary have developed.

Warming (1870) (69) is confident that it is not the independent foliar appendage, for ontogenetic proof, analogy to other genera, and the course of vascular strands indicate that it is a foliar organ which is closely allied to the involucre. He designates it as a stipule serving as a nectary without morphological independence.

Bentham (1880) (6) considers the glands to represent lateral glands of the involucral bracts, fused in pairs with the fusion of the bracts in the formation of the gamophyllous involucre. Two contiguous glands of adjoining bracts unite into a single two-lobed gland, and the glands alternate with the lobes of the involucre.

Before discussing the anatomy of the gland it may be well to give a *résumé* of the morphological situation of the species studied. In some species the gland is composed of two parts—one thickened and glandular, the other petaloid, the vascular supply of both arising after that of the involucral bracts. On this basis the species may be divided into two groups—those possessing petaloid appendages to the gland, as in *E. fulgens* (Fig. 62, *g*) and *E. marginata* (Fig. 1), and those in which these structures are entirely absent, as in *E. Esula* (Fig. 5) and *E. Cyparissias.*

Except in *E. splendens* the vascular supply for the gland arises from each primary axis after the departure of the foliar trace to the involucre. *E. pulcherrima* gives definite proof that each stele supplying the gland originates as a double strand forming a ramular trace of a distinct siphono-stelic nature (Fig. 36, *e, e'*). Since in primitive species like this the number and method of origin of the traces are those of a branch, and in subsequent behaviour a definite stele is formed, it is evident that the supply to the gland is a branch supply. However, in the majority of species reduction has gone so far that the two traces have become one, and the steles have become contracted, losing their pith and becoming concentric bundles. These ramular traces gradually migrate outward and upward to the level where the bracts are freed one from the other. Where glands are present these traces undergo further segmentation. This is true particularly in the glands of Group III. In these species two adjacent contracted axial cylinders continue for a short distance towards the gland, and then by tangential division increase in extent of tracheae in such a manner as to form a ring or stele of vascular tissue (Fig. 19, *g, g'*). Coincident with the formation of this stele is the formation in some species of secondary branches of the lateral leaf traces of the bract. These also pass into the gland (Fig. 42). Thus in some cases the vascular supply of the gland is augmented by contiguous rami of lateral foliar traces of the two adjacent bracts (Fig. 49). All these vascular strands end near or in contact with large storage cells, which lie about two or three cells below the palisade

secreting layer. Hence the stele, much contracted at its origin, becomes, together with the adjacent stele, and in some cases bract bundles, a very highly complex vascular ring. In the case of *E. pulcherrima* reduction in the number of glands has reached its climax, and one elaborate gland is formed. In this gland the formation of vascular tissue simulates a distinct stele, as in other glands, but a second irregular tangential and radial division gives rise to a second ring concentric to the first (Figs. 45 and 46). Thus a vascular supply to the gland, which is even more complex, is formed.

In species where petaloid appendages are developed, a second ring of vascular tissue does not form. The appendages are supplied by the first-formed branches of the ramular traces, and the gland traces are branches from this main trace.

All anatomical evidence leads to the theory that the glands are fundamentally highly differentiated secondary ramular structures, the primitive function of which was not secretory. The specialized function was brought about by entomophily. This elaborate structure includes not only the vascular traces of two branches, but the contiguous traces of the adjacent involucral bracts. Evidently great suppression, coupled with change of function, caused the fusion of a whorl of alternate bracts and pairs of branches, and the formation of the gamophyllous ‘involucre’. Each pair of branches and the adjacent bract bundles become the vascular system of the new structure, the gland borne on the margin of the involucral cup.

The Pistillate Flower.

The exserted pistillate flower which terminates the main axis of the condensed inflorescence within the gamophyllous involucre is generally well developed, though rudimentary ones may occur in some species. In all species observed the pistillate flower was composed of three carpels, a single short style, and three stigmatic surfaces which may be dissected, fimbriated, flat, or ridged. The position of the carpels with relation to the bracts is definite. One carpel is superposed to the third bract, another between 1 and 4, and a third between involucral bracts 2 and 5. In some instances where only four glands are present there seems to be a tendency towards the abortion of the carpel between the bracts 2 and 5.

The one feature of the pistillate flower which has been the subject of much controversy is the disc at the base of the ovary. In the majority of the species examined this structure apparently resembles a mere hypertrophy of the terminus of the pedicel, that is, the axis. In two species, *E. clusiaefolia* and *E. hexagona*, three small scales arise in this region. Each carpel is superposed to one of these scales.

Payer (1857) (52) believed the disc to be a swelling of the axis much as occurs in other plants. He held that its development, after the formation of the ovary, prevents one calling it a calyx, for all calyces arise before the pistil. Baillon (1858) (2) recognized it as the disc homologous with the calyx. A. L. de Jussieu (1789) (39), Lamarck (1786) (42), R. Brown (1818) (18), Roeper (1824) (59), Wydler (1845) (76), Schmitz (1871) (63), and Warming (1870) (69) follow the opinion of Baillon, that the disc is a calyx. Müller (1872) (48), from his investigations, expressed the belief that several species verified its calycine origin. Čelakovsky (1872) (21) believes it is the region where leaflets may grow, or where a leaf whorl is suppressed, therefore it represents a suppressed perigonal whorl. Schmidt (1906) (62) regards these structures as vestiges of a late or abortive perigon. Bower (1908) (13) states that 'the pistil is composed of three coherent carpels with a rim below which represents an abortive perigon. This justifies the conclusion that here is a very advanced state of meiomery. Such extreme reduction usually is connected with a close crowding of numerous flowers in an aggregated inflorescence.'

Anatomical investigations show that this structure lacks vascular strands. The discs observed consist of modified epidermal and hypodermal tissue, more or less homogeneous, without any associated strands of adjacent organs. Since this region bears appendages which can be seen in all stages of degeneracy until they have vanished completely, leaving the nodal region only, this latter organ may be considered an abortive perianth whorl, in which the disappearance of its individual members is closely correlated with the disappearance of the vascular elements.

If the disc represents a rudimentary calyx beneath the well-developed ovary, the pistil must be interpreted as a single flower upon a distinct pedicel, which, in this case, represents the main axis of the cyathium. The vascular supply in the pedicel is organized, in some cases, as a distinct siphonostele which gives rise only to the carpellary supply at the base of the ovary. In other instances the carpellary supply is well defined for a considerable distance in the pedicel as carpellary strands, or as dorsal carpellary and marginal carpellary traces.

Thus the anatomical evidences seem to prove that this pistillate flower is a distinct one with an abortive calyx, situated on a distinct pedicel, which represents the main axis of a cymose inflorescence.

The Phyletic Rank of the Subgenera of the Genus.

The question now may arise as to whether or not anatomical evidence can throw light upon the phylogenetic relationship of the subgenera of the genus *Euphorbia*. It may be suggested that from an anatomical point of view it does seem that perhaps the genus may be divided into two sections,

based upon the types and behaviour of the steles.   De Candolle (1866) (23) has made twenty-six sections which are very unequal in systematic value and number of species.   Engler and Prantl (1890) (27) have divided the genus into six sections—*Anisophyllum, Adenopetalum, Poinsettia, Eremophyton, Euphorbium*, and *Tithymalus*, based upon the phyllotaxy, the position of the cyathia, and the presence or the absence of petaloid appendages.

The anatomical studies reveal the fact that the sections *Adenopetalum, Poinsettia*, and *Euphorbium* have slight affinities, while *Anisophyllum* and *Tithymalus* are more closely related.   Where the section *Eremophyton* belongs the writer is not prepared to say, as species of this section were not available for study.   By some authors *Anisophyllum, Tithymalus*, and *Poinsettia* are often considered distinct genera, but the characters are solely derived from habit, and not sufficiently definite for generic separation.

GENERAL DISCUSSION.

The anatomical study has revealed definite proof that the ' flower ' is an inflorescence, and has shown among the species investigated an order of succession which exemplifies evolutionary tendencies within the genus. That the flower is not an hermaphroditic one is very obvious.   The flower is to be considered a cyathium composed of a very highly specialized inflorescence, including primary, secondary, and tertiary branches, all of which are traversed by normal or specialized siphonosteles.

The following conditions favour the idea that the euphorbian inflorescence 'flower' is itself very highly specialized, and not simple or primitive : the tendency for alternate arrangement of the first and secondary rami to become opposite and whorled ; the sequence from spiral order of departure of the inflorescence branches from the main axis to the reduction and obliteration of this phyllotaxy in the subsequent branching ; the atrophy of tertiary branches and the gradual reduction in the number of functional rami ; the decrease in the number of glands ; the presence or the absence of petaloid appendages ; the reduction of the entire bracteole ; the reduction of floral parts of the single flower to a single stamen, and a suggestion of a perianth ; the reduction of the pistillate flower to three uniovulate carpels and the disc which represents an abortive perianth without vascular supply. These conditions emphasize specialization through reduction.

This extreme complexity and congestion, as well as evolutionary reduction within the involucral bracts, has doubtless resulted from a suppression of nodes and internodes, aggregation of branches, elaboration of glands, cohesion of bracts, abortion of bracteoles, unequal development of the lateral branches of the dichasium, and reduction of the individual flowers to naked monandrous flowers.

Within the limits of the species observed there is reduction in the vascular tissue of the peduncle; a reduction in the tissue of the siphonostele; in the size of the inflorescence; in the number and the size of the glands; in the vascular strands to the gland; in the number of monandrous flowers and consequently of stamens; and lastly, in the extent of the bracteoles.

Considering the individual species, some represent more primitive types, while others show specialization in certain other features. *E. clusiaefolia* with numerous stamens, five glands, and the entire bracteoles, probably approaches the seemingly primitive condition within the genus. *E. pulcherrima*, on the contrary, with its atrophied bracteoles, its reduction of glands to one, makes this one of the more highly evolved types. Here also is the specialization and elaboration of this one gland and the absence of petaloid appendages. *E. Esula* represents the extreme of reduction, possessing but a single stamen in each primary inflorescence, and a consequent limitation in all vascular tissue.

The species in Group I seem to show a greater specialization in some features accompanied by reduction in a few structures such as the bracteoles, tertiary branches, and gland. The one common character is the well-organized siphonostele and its entire branching system.

In Group III there seems to be a greater uniformity in the presence and behaviour of the concentric or modified steles. The monandrous flowers have been reduced from thirteen to one, while the glands remain four or five in number.

SUMMARY.

I. The anatomy of the 'flower' of the genus *Euphorbia* discloses a number of morphological features extending throughout the genus which are not revealed from macroscopic investigations.

1. The 'flower' of *Euphorbia* represents an inflorescence in an advanced stage of reduction.

2. The inflorescence is cymose, and is composed of a central axis and five main branches which arise from the main axis in spiral sequence, 2/5 phyllotaxy (Fig. 9).

3. The entire inflorescence is compressed within a gamophyllous 'involucre'. This 'involucre' comprises a whorl of alternate bracts and pairs of secondary branches of the inflorescence branch (Fig. 17, b, g, g').

4. Each involucral bract subtends an inflorescence branch and is supplied with a single foliar trace. This single foliar trace represents the fusion of the usual three foliar traces of a leaf of *Euphorbia*. In the upper part of the bract these traces remain free (Fig. 51, b, b^2, b^3).

5. Each primary section of the inflorescence branch is composed of a dichasium and two monochasia (Fig. 15, *R*, *L*, *p*). The dichasium is supplied with normal steles ; the monochasia with specialized reduced steles. The presence of these steles is proof of the branch nature of these ' flower ' parts.

6. The branches which give rise to the three oldest flowers form a dichasium ; each of the lateral branches of this dichasium may form a monochasium (Fig. 15).

7. Each monochasium bears a series of monandrous flowers which vary in number in different species, seven to one (Figs. 90–4). The mono-chasium is absent when the number of staminate flowers in the entire primary section is reduced to three or less (Figs. 97–9).

8. Each gland represents a pair of modified secondary branches of a lateral inflorescence branch, fused with which are parts of the adjacent involucral bracts (Fig. 51).

9. Petaloid appendages are closely associated with the gland, and the two structures are apparently morphologically parts of a single structure (Figs. 57–62).

10. The development of the gland has been acquired as an accompaniment of flower reduction, and of development through an entomophilous habit.

11. Each monandrous flower consists of a single stamen situated upon a pedicel, the vascular tissue of which represents a pithless stele. The articulation which occurs between the pedicel and the filament of the flower represents the position of an abortive perianth (Fig. 6, *G*).

12. The monandrous flowers may be subtended by vestigial (Fig. 40, *z*) or well-developed bracteoles lacking vascular tissue (Fig. 17, *z*). When present each bracteole arises at the base of the pedicel of the monandrous flowers (Fig. 50, *z*), but in some cases is fused to the base of the pedicel. The bracteoles are variable in size, shape, and extent (Figs. 100–12). Contiguous bracteoles become fused, and those of the adjacent primary inflorescences form an involucral partition or commissure.

13. The ' ovary ' of the cyathium is a pistillate flower which terminates the main axis of the entire cyathium (Fig. 2, *P*). The pistil is composed of three uniovulate carpels, with a disc beneath the ovary. The disc represents an abortive perianth.

II. Coincident with the reduction of the individual flower has gone aggregation, suppression, and cohesion in the cyathium. The reduction in the ' flower ' consists in the disappearance of the inner branches of the dichasium and the inner branches of the monochasium (Figs. 90–9).

III. The flowers and reproductive axes of the Euphorbiaceae afford an excellent example of the disappearance of the vascular system in advance of the organs which it supplies. The vascular supply may persist as

abortive elements in the secondary steles long after the branches have become obliterated.

IV. The evidence revealed by anatomy shows that the genus *Euphorbia* is very highly specialized; a fact which morphology, ontogeny, and teratology verify and uphold.

ACKNOWLEDGEMENTS.

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The Ontogeny of Graviperception in Osmunda regalis.

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With three Figures in the Text.

THE study of ontogeny has concerned itself almost entirely with the development of the organs and tissues of plants and animals. In the case of a fern frond, it has been shown (4 and 7) that the young organ, in its increasing sensitiveness to gravity, develops physiologically as well as morphologically ; and it is the chief purpose of the present work to demonstrate that this sensitiveness also undergoes development in the plant as a whole, i. e. in successive years.

The methods employed are those fully described by Miss Waight in her work on *Asplenium bulbiferum* (7), and consist briefly in ascertaining the presentation time under constant conditions for a response to gravity at different stages in the development of the frond, and of the plant as a whole. The plants were grown in the same greenhouse, provided with blinds, hygrometer, maximum and minimum thermometer, and a thermo-regulator, which latter usually kept the temperature round about 20° C. The experiments recorded in this paper were performed at 20° C. ± 1°, and at a relative humidity as near 80 per cent. as possible. Most experiments were carried out in diffuse daylight, since it was desired to study the plants under natural conditions, but light effect was always considered, as previously explained (4), and many control experiments were made on the smaller plants in the dark, other conditions being identical. These gave entirely similar results.

As previously defined (7, p. 57) the presentation time is that period ' which, under the given conditions, will generally produce a movement of about 5° and only very rarely exceeding 10° '. Individual variation is too great to permit a more precise definition, though such variations can of course be treated by the usual mathematical methods (cf. 6). But the

movement of a fern frond cannot be read like that of a galvanometer needle, since no two fronds are alike, and they are moreover capable of executing movements other than those due to gravity (see 4). Whether therefore a geotropic movement has taken place or not, and in the former case the amplitude of such movement, is best judged by the observer trained by many hundreds of experiments on the material in question.

The expression 'latent time', i. e. 'the period elapsing between the beginning of stimulation and the first indication of response', is used as in two former papers of this series (4, p. 146, and 7). It is preferred to the 'reaction time' of some German writers as being more explicit, since the whole reaction includes further phenomena than those just defined.

The following abbreviations are used for the different stages of the frond's development :

E. I. = Early infant, i. e. very young fronds of only a few days growth, leaf-
lets small and in the inner coils. (N.B.—The average length in cm.
of these and other stages is given in the corresponding tables.)

M. I. = Middle infant, a stage about half-way between E. I. and L. I.

L. I. = Late infant, i. e. leaflets still enclosed in apical coil, but first pair
visible, and directed either horizontally and abaxially, or down-
wards.

A. 1, 2, 3, &c. = Adolescent 1, 2, 3, &c., i. e. 1st, 2nd, or 3rd, &c. pairs of
leaflets expanding and flush with the apical coil.

Mo. = Sub-mature, i. e. the apical coil is uncurled, but the free tip is point-
ing inwards, or adaxially.

M. = Mature, i. e. fully expanded—the upper part of the rachis is either in
a straight line, or curved more or less backwards or abaxially.

M. 1, 2, 3, &c. = Mature for 1, 2, 3, &c. days.

In addition : P.S. = period of stimulation, P.T. = presentation time, and L.T. = latent time.

The term 'adult' has been employed throughout for the oldest plants, used to avoid confusion with 'mature', applied to the fronds.

The work on adult plants will first be given. These were supplied by Messrs. Waterer of Twyford, and were 1½–3 ft. in height, one plant bearing a frond, two pinnae of which were fruiting. Experiments were made on these plants throughout the summer of 1923 ; in the autumn, when growth ceases, they were put in the ground in the open and covered during the winter.

In the early spring when young fronds appeared, the pots were dug up, and replaced in the greenhouse. The fronds grew rapidly, many fruiting abundantly, i. e. they bore sporangia on the whole of the upper reduced pinnae in the normal manner. This showed that the plants were thoroughly healthy, and fresh sets of experiments were made, supplementing and con-

firming those of the previous year. Some representative experiments, together with the deduced presentation times and latent times, are given in tabular form as follows :

Adult Plant.

Length of Frond in cm.	P.S. in Minutes.	Angle of Curvature.	L.T. in Hours.
Stage : E. 1 = 2·6 cm.			
2·5	150	5°	8
2·5	150	5°	$5\frac{1}{2}$
1·9	150	3°	5
2·0	150	—	—
3·0	120	—	—
3·6	120	—	—

P.T. = $2\frac{1}{2}$ hours; L.T. = $6\frac{1}{3}$ hours.

Stage : L. 1 = 13·3 cm.			
10·4	8	5°	2
12·4	7	5°	$1\frac{1}{4}$
15·2	5	—	—
13·5	5	—	—
15·2	4	—	—

P.T. = 7 minutes; L.T. = $1\frac{3}{4}$ hours.

Stage : A. 1 = 14·8 cm.			
15·6	6	5°	$1\frac{1}{2}$
12·6	6	5°	$1\frac{1}{4}$
19·5	5	—	—
10·9	4	—	—
9·0	3	—	—
21·2	2	—	—

P.T. = 6 minutes; L.T. = $1\frac{1}{2}$ hours.

Stage : A. 2 = 15·9 cm.			
18·7	10	15°	$1\frac{1}{6}$
11·5	8	10°	$1\frac{1}{4}$
22·0	5	5°	$1\frac{1}{2}$
8·6	5	5°	2
8·2	5	5°	$1\frac{1}{2}$
25·0	4	—	—
17·6	4	—	—

P.T. = 5 minutes; L.T. = $1\frac{1}{2}$ hours.

Stage : A. 3 = 21·3 cm.			
19·4	5	4°	$\frac{1}{2}$
22·6	3	3°	$1\frac{1}{2}$
21·0	3	10°	2
15·3	3	10°	2
24·9	2	—	—
24·8	2	—	—
21·0	2	—	—

P.T. = 3 minutes; L.T. = $1\frac{1}{2}$ hours.

Stage : A. 4, 5, and 6 = 33·2 cm. (Number of pairs of leaflets unfolded placed in brackets after length in this and subsequent tables).

27·2 (4)	1	5°	2
24·7 (4)	1	10°	$1\frac{1}{4}$
20·8 (4)	1	5°	$1\frac{1}{4}$
14·6 (4)	1	4°	$\frac{1}{4}$
59·0 (6)	$\frac{1}{2}$	—	—
53·7 (5)	$\frac{1}{2}$	—	—
32·7 (4)	$\frac{1}{2}$	—	—

P.T. = 1 minute; L.T. = $1\frac{3}{4}$ hours.

Length of Frond in cm.	P.S. in Minutes.	Angle of Curvature.	L.T. in Hours.
Stage: Mo = 38·2 cm.			
61·5 (7)	1	10°	1¼
57·5 (6)	1	3°	1¼
22·4 (8)	1	3°	1¼
33·0 (7)	½	10°	2
29·0 (5)	½	10°	1
18·3 (6)	½	30°	1
42·5 (6)	½	—	—
41·5 (8)	½	—	—

P.T. = 1 or ½ minute ; L.T. = 1¼ hours.

Stage : M. = 44·4 cm.			
25·5 (7)	1	5°	2
22·0 (7)	1	20°	1¼
69·5 (10)	½	—	—
61·5 (10)	½	—	—

P.T. = 1 minute ; L.T. = 1⅔ hours.

Stage : M. 1 = 44·9 cm.			
25·0 (7)	1	15°	3
18·7 (6)	1	8°	¾
69·5 (10)	½	—	—
66·3 (10)	½	—	—

P.T. = 1 minute ; L.T. = 1⅞ hours.

Further experiments on mature fronds of various ages have shown that they retain their power of response to one minute's stimulation for a day or two, but after this sensitivity gradually declines. Thus an M. 3 responded to three minutes' stimulation, one M. 4 to four minutes', another similar M. 4 did not, while an M. 5 was unresponsive to eleven minutes' stimulation.

In the course of the work it was found that the first two or three fronds of the season gave lower presentation times in their earlier stages than later ones, and these results have not been included in the above table. This increased irritability may be due to a special spring exuberance, or the slightly artificial conditions may later adversely affect the perceptive power, for the plants had to be grown in pots although every effort was made to keep them in as natural conditions as possible. However, no difference in the behaviour of the fronds was shown in their later stages, i. e. A. 4 &c. ; and even if the irritability is somewhat greater than that deduced from the above figures, this would only emphasize the general conclusions drawn. Experiments where no movement took place have also been neglected wherever it was subsequently found that the frond had not grown, for though geotropic curvature is possible, it rarely occurs unless the organ is actually growing during the latent period.

In the autumn of 1921 and of 1922 spores were sown in pots kept in the greenhouse. On germination they produced quantities of prothallia, each crop giving rise to sporophyte plants in the following spring. Some sensitivity to gravity and power to respond is probably present from the first in the immature leaves of the young sporophyte, though chiefly, owing

to the minuteness of the organs, it is difficult to demonstrate. In one case the seventh and eighth leaves while still immature curved completely upwards, i. e. through 90°, with continuous stimulation in twenty-four hours or less. The results shown in Table I were mostly obtained from plants in June and July after the prothallia had withered. The leaves when fully grown were about 3 or 4 cm. long and were usually either three-lobed, or possessed two leaflets and a terminal lobe. The stage described as A. 1 is that in which the two lateral leaflets, or the two lobes of the undivided blade, are horizontally directed adaxially.

TABLE I.

Sporeling Plant—Year I.

Length of Frond in cm.	*P.S. in Minutes.*	*Angle of Curvature.*	*L.T. in Hours.*
Stage: E. 1 = 4·6 mm.			
0·55	300	5°	9
0·5	300	12°	7
0·45	300	5°	7
0·4	300	—	—
0·5	270	5°	7
0·4	240	—	—
0·45	210	-	- -
0·4	180	—	—
		P.T. = 5 hours; L.T. = 7½ hours.	
Stage: M. 1 = 0·8 cm.			
0·85	120	10°	3½
0·8	120	3°	4
0·7	120	3°	3
0·6	60	—	—
0·5	30	—	—
1·4	20	—	- -
		P.T. = 2 hours; L.T. = 3½ hours.	
Stage: L. 1 = 1·6 cm.			
1·5	30	6°	2¼
2·5	25	15°	1½
1·5	25	15°	2¼
2·0	20	4°	1½
1·1	20	4°	5
2·0	20	—	-
1·7	20	—	—
1·4	15	—	—
		P.T. = 25 minutes; L.T. = 2⅗ hours.	
Stage: A. 1 = 2·6 cm.			
2·5	30	13°	3½
1·8	30	10°	2
3·3	20	7°	2½
3·4	20	—	—
2·5	20	—	—
2·2	20	—	—
		P.T. = 25 minutes; L.T. = 2⅔ hours.	

Length of Frond in cm.	P.S. in Minutes.	Angle of Curvature.	L.T. in Hours.
Stage : M. = 2·8 cm.			
3·2	30	20°	2
2·0	30	15°	2
3·6	20	3°	$1\frac{1}{2}$
3·6	20	10°	3
2·5	20	5°	$1\frac{1}{2}$
2·2	20	—	—
2·4	15	—	—

P.T. = 20 minutes ; L.T. = 2 hours.

Stage : M. 1 = 3·3 cm.			
4·5	70	25°	$2\frac{3}{4}$
2·3	60	10°	4
2·3	50	15°	$3\frac{1}{2}$
3·4	30	5°	2
4·7	30	—	—
2·5	30	—	—

P.T. = 40 minutes ; L.T. = 3 hours.

In the autumn these first-year plants ceased growing. They were kept in the greenhouse throughout the winter, growth recommencing in the spring of 1923. When fully developed, the fronds were about 10 cm. long, each with a terminal leaflet, simple or three lobed, and usually three or four pairs of leaflets, two pairs of which were usually more or less deeply lobed. They never fruited, though single fronds in several cases bore sporangia in 1924, i. e. in the third year of life.

Table II gives the results of similar experiments on these second-year plants.

TABLE II.

Length of Frond in cm.	P.S. in Minutes.	Angle of Curvature.	L.T. in Hours.
Stage : E. I = 0·63 cm.			
0·6	240	10°	$7\frac{1}{4}$
0·8	180	5°	$6\frac{1}{2}$
0·6	180	8°	$6\frac{4}{5}$
0·45	180	3°	6
0·6	150	5°	6
0·7	150	—	—
0·65	150	—	—
0·65	150	—	—

P.T. = 3 hours ; L.T. = $6\frac{3}{4}$ hours.

Stage : M. I = 1·2 cm.			
1·35	60	5°	$2\frac{3}{4}$
1·25	60	10°	2
1·15	45	5°	$2\frac{1}{4}$
1·0	45	5°	$2\frac{1}{2}$
1·25	30	—	—
1·2	30	—	—

P.T. = $\frac{1}{2}$ hour ; L.T. = $2\frac{1}{2}$ hours.

Length of Frond in cm.	P.S. in Minutes.	Angle of Curvature.	L.T. in Hours.
Stage: L. I = 2·8 cm.			
3·6	18	5'	2
2·9	18	10°	2¾
1·4	18	3°	2
3·8	17	—	—
2·5	17	—	—

P.T. = 18 minutes; L.T. = 2¼ hours.

Stage : A. 1 = 5·5 cm.			
3·2	17	5°	2½
3·8	15	2°	3
5·0	15	10°	1½
8·0	12	—	—
7·0	12	—	

P.T. = 15 minutes; L.T. = 1⅓ hours.

Stage : A. 2 = 7·5 cm.			
5·5	13	3°	3
7·4	12	5°	1¼
7·0	12	10°	1¼
6·1	12	5°	2½
7·2	12	—	—
4·8	12	—	..
9·7	11	—	—
12·0	10	—	..

P.T. = 12 minutes; L.T. = 2 hours.

Stage: M. = 8·4 cm. (Number of pairs of leaflets unfolded placed in brackets after length in this and following table).

9·2 (3)	5	30°	2½
4·9 (1)	5	10	2
12·2 (4)	4	5	2 3/10
11·0 (4)	4	10	1
4·6 (3)	4	3	1⅛
7·9 (⅕)	3	5	1
7·3 (2)	3	5	1¾
10·5 (4)	3	—	—

P.T. = 3 minutes; L.T. = 2 hours.

Table III illustrates the gradual loss of sensitivity after maturity has been reached, but the experiments are insufficient for a deduction of the P.T. and L.T.

TABLE III.

Stage : M. 1			
12·0 (4)	4	5	2¾
8·0 (2)	4	—	—
4·0 (3)	4	—	—
Stage : M. 2			
13·1 (4)	10	20°	2
5·0 (3)	10	5°	2
Stage : M. 5			
15·0 (4)	75	10°	2½
6·5 (3)	25	—	—

It should be noted from the above tables, that when a frond is mature it attains the increased degree of sensitivity independently of the

number of leaflets it bears (cf. *Asplenium bulbiferum*, 7, p. 59); e. g. A. 2 requires at least twelve minutes' stimulation for a visible response, while four minutes is sufficient for fronds of similar length and number of leaflets when it is completely uncurled.

A summary of the results shown in the previous tables is given in Table IV. The semi-diagrammatic sketches (Figs. 1–3) of the fronds indicate their relative size and morphological development at maturity in the different years. The age of the adult plants experimented upon is not known, but is probably not less than five or six years. One of them, considerably smaller than the others, though bigger than those of Year II, scarcely ever responded to the period of stimulation sufficient for the other plants; i. e. in both morphological and physiological development it came between Year II and adult plants. The fifth column gives the average relative sensitivity of the frond at its different stages for each year, calculated from the reciprocals of the corresponding presentation times.

TABLE IV.

Stage of Plant.	Stage of Frond.	Length of Frond in cm.	P.T. in Minutes.	Sensitivity.	L.T. in Hours.
Year I.	E. I	0·46	300	2	7·5
	M. I	0·8	120	5	3·5
	L. I	1·6	25	24	2·8
	A. 1	2·6	25	24	2·7
	M.	2·8	20	30	2·0
	M. 1	3·3	40	15	3·0
Year II.	E. I	0·63	180	1	6·6
	M. I	1·2	45	4	2·9
	L. I	2·8	18	10	2·25
	A. 1	5·5	15	12	2·7
	A. 2	7·5	12	15	2·0
	M.	8·4	3	60	2·0
Adult.	E. I	2·6	150	1	6·15
	L. I	13·3	7	21	1·75
	A. 1	14·8	6	24	1·38
	A. 2	15·9	5	30	1·5
	A. 3	21·3	3	50	1·5
	A. 4–6	33·2	1	150	1·38
	Mo.	38·2	1 or }	150 or 300	1·25
	M.	44·4	1	150	1·63

DISCUSSION OF RESULTS.

The most obvious fact, shown by a glance at Table IV, is that the sensitivity alters with the stage of development of both the frond and the plant. It is also evident that in the individual frond gravitational irritability increases in much greater ratio than growth; for even in the sporeling plant, whereas the ratio of the frond lengths from E. 1 to M. is not quite 1 : 6, the sensitivity to gravity is 1 : 15, while the disparity is far greater in the older plants.

In the ontogeny of the plant, however, the physiological property does not usually keep place with morphological development for corresponding stages of the frond in the infant phase. It is approximately equal during adolescence, and far surpasses it at maturity. This is made clear from a study of the following table:
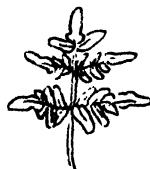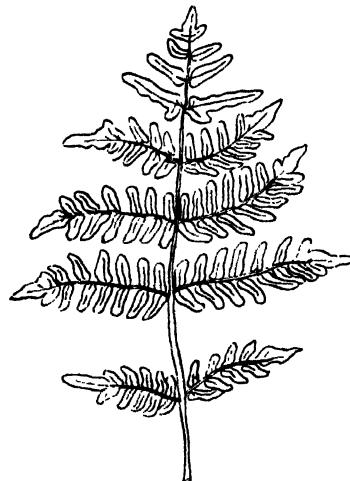
FIG. 1.   FIG. 2.   FIG. 3.

TABLE V.

Stage of Frond.		Year I.	Year II.	Adult.
E. 1	Length	1	1·4	6
	Sensitivity	1	1·7	2
M. 1	Length	1	1·5	
	Sensitivity	1	2·7	
L. 1	Length	1	1·8	8·3
	Sensitivity	1	1·4	3·6
A. 1	Length	1	2·0	5·7
	Sensitivity	1	1·7	4·0
A. 2	Length		1·0	2·1
	Sensitivity		1·0	2·0
M.	Length	1	3·0	13·6
	Sensitivity	1	6·7	20 or 40

It may further be of interest to compare *Osmunda regalis* with other plants in regard to behaviour to gravity. The only other member of the Pteridophyta, so far as I know, in which corresponding facts have been ascertained is *Asplenium bulbiferum*. We find general resemblances between the two ferns in the decrease of the presentation time and the latent time from the infant to the adolescent stage; its rise after the minimum has been reached, and the cessation of all irritability before growth ceases. Again, though periods of stimulation considerably higher than the presentation

time do not necessarily give different results from the latter, they tend to increase the angle of curvature, and, though less often and to a slighter extent, to decrease the latent time.   This would be more fully apparent if all the experiments had been given, but it is illustrated in the tables above: Adult, A. 2; Sporeling I, A. 1 and M.; Sporeling II, M. 1 and M., and also in the experiments on *Asplenium* (7).   The length of the frond hardly affects the presentation time at all, as is very clearly shown in the tables for both ferns.   It may also be noted that at their most sensitive stages, a period of stimulation cannot always be found which will elicit only a slight response on the part of either fern ; they will execute fairly big movements (10°–30°), or they will not curve at all—i. e. they tend to obey the 'all or none' law of animal physiology.   Very few examples of this seem to be known for plants, though Bose (2) records an illustration in the response of *Biophytum sensitivum* to electrical stimulus.

In both cases the figures for the latent time show that it is a more individually variable[1] quantity than the presentation time, though its extremes have a far lower ratio, being only 1 : 3·2 for *Asplenium* and 1 : 5 for *Osmunda*, whereas the ratios for the presentation time are 1 : 16 in the former case and 1 : 150 (or 300) in the latter.   It is practically constant in both ferns for the stages A. 1 to A. 8.

More interesting, however, is the physiological distinction between the two genera, which expresses itself in the following ways :

1. The most sensitive stage of the frond in *Asplenium* is at A. 5–7, while in *Osmunda* it occurs at maturity—a stage at which all geotropic irritability has ceased in the former case.

2. The greater ratio in the presentation times at early and late stages is pointed out above, and it may be noted that the growth ratios for the same stages are nearly the same, i. e. 1 : 13 or 1 : 14.

3. In their most sensitive conditions *Osmunda* fronds are thirty (or sometimes even sixty) times as sensitive as those of *Asplenium*.

4. For adolescent stages the L.T. ratio is 1 : 3·5, i. e. *Osmunda* responds three and a half times as quickly as *Asplenium*.

In comparing *Osmunda* with plants of other phyla some difficulty arises from the fact that the records do not always mention all the relevant factors. Czapek (3), using chiefly seedlings and sporophores of *Phycomyces nitens*, never found a lower presentation time than fifteen minutes.   Several workers have since recorded much shorter periods ; the lowest I have been able to trace for an Angiosperm being two minutes for the inflorescence axis of *Capsella bursa-pastoris* (1).   Streeter gives one minute as sometimes

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[1] The extremes of this variation are, however, hardly ever greater than 1 : 2 and usually much less.   The L.T. for all adolescent stages of *Osmunda* is almost invariably between 1 and 2 hours, Waight finds a lower ratio for *Asplenium*, while Tröndle has a range of 1 : 6 or even 1 : 9 for cress seedlings with centrifugal force.

sufficient for the sporophores of *Amanita*, and half a minute as yielding no response (5). But only a few experiments were made on the presentation time, and experimental conditions, even the important one of temperature, are not mentioned, so that no entirely satisfactory comparison can be made. It would therefore appear that the shortest presentation time yet recorded for any plant for response to gravity is one or even half a minute for almost mature fronds of *Osmunda regalis*, though several plants are known to have much lower latent periods. But the latter quantity must depend at least in part on the motor mechanism of the plant, while the presentation time is probably the best measure we have of the irritability of an organ to a given stimulus. So we may consider the royal fern as the most sensitive plant to the force of gravity as yet discovered, and hence deserving its popular name on physiological as well as morphological grounds.

SUMMARY.

1. The physiological property of irritability to gravity is shown to undergo ontogenetic development to a much greater extent than morphological development in both the individual frond and the whole plant of *Osmunda regalis*.

2. In presentation time and latent time certain general likenesses are shown to the corresponding phenomena in *Asplenium bulbiferum*; but in important details the two genera are as distinct physiologically as they are morphologically.

3. *Osmunda regalis* appears to be more sensitive to gravity than any plant yet recorded, since its fronds, when just mature, will respond to one or even half a minute's stimulation.

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The Effect of Length of Day upon the Growth and Reproduction of some Economic Plants.

BY

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With Plates XVII-XIX.

INTRODUCTION.

REVIEWING the position of Agricultural Research in his Presidential Address to Section M, Agriculture, at the Toronto meeting of the British Association, Sir John Russell (29) has recently pointed out that the period of agricultural research since 1884 has been marked by the recognition that the plant is plastic. During this period economic problems concerned with plant growth as affected by inherent and environmental factors have been subjected to extensive investigation.

On one hand, the hereditary factors have been subjected to such very thorough investigations by geneticists, while on the other hand, by controlling the environmental conditions and so regulating growth, the plasticity of economic plants has been further demonstrated.

The work of Klebs (22), and more recently that of Garner and Allard (7), to quote but two examples, has shown that plant growth and reproduction can be controlled by manipulation of the environmental factors.

Garner and Allard have been able to control flowering and to modify vegetative growth by altering the period of daily illumination. Not only did they curtail the period of illumination, they also carried out series of experiments with artificial light, to prolong the natural period of illumination. An attempt has been made at the Welsh Plant Breeding Station to confirm part of this work done in America, under the conditions

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prevailing in Cardiganshire. Particular attention has been paid to the behaviour of different strains of herbage plants, whose hereditary characteristics are under investigation.

In the first season's experiments, it was thought desirable to investigate the behaviour of as many types as possible under normal and shortened daylight periods. For this purpose plants grown in boxes or pots placed on trucks were run in and out of a large three-sectioned hut, so that the different series received twelve, nine, and six hours' daylight. The plants were out in the open air from 6 a.m. to 6 p.m. (G.M.T.) for the twelve-hour series; from 9 a.m. to 6 p.m. for the nine-hour series; and from 12 noon to 6 p.m. for the six-hour series. Control plants were grown in similar pots and received the full natural daylight of the season.

The hut was specially constructed to aid ventilation, whilst preventing light from entering. Thermometers, one in each section, rarely showed a difference of 3° F., or more, between the temperature of a dark closed section with the plants inside and that of the control plants outside.

Equal soil and water conditions were provided as far as possible. In practice this amounted to keeping the soil of plants under treatment as moist as the soil of the control plants.

RESULTS OBTAINED.[1]

It will be convenient to group the results obtained into five divisions, namely :

I. *Ever-blooming Type.* Plants in this division flowered equally well under all the four periods of daylight; full day control, twelve, six, and nine hours.

II. ' *Short-day* ' *Plants.* Plants placed in this division are characterized by the fact that shortening the light period hastens flowering.

III. ' *Long-day* ' *Plants.* Plants placed in this group flower earlier when they receive the full illumination; shortening the light period prevents or delays flowering.

IV. *Intermediate types.*

V. *Results with types that did not flower.*

Division I. Ever-blooming Types.

Poa annua.—Annual Meadow Grass.

Twelve equal small plants obtained from the immediate neighbourhood were subjected to each treatment from 1st February. By the 3rd March all four series contained plants with emerging panicles. By the 25th March almost every plant had produced fully open panicles. Seed production was normal in the four series.

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[1] Preliminary Notes (32) on these results were published in Nature, vol. cxiv, p. 350, Sept. 6, 1924.

Division II. Short-day Plants.

1. *Phascolus multiflorus* (Runner Bean—Sutton's 'Best of All'). Ref. No. As. 74. (Photographs, Plate XVII, Figs. 1–5.)

Eight equal small plants raised from seed sown 7th May were subjected to the four light periods from 31st May 1924. Whilst the control plants grew normally, producing rapidly elongated stems, the treated plants remained short. On 31st July the control plants were 42 in. high, twining round a support, the 12-hours plants were 10 in. high, and the 9- and 6-hours plants only 9 in. high. All the plants subjected to the shorter days produced thick swollen nodes ; the axillary buds of the first leaves developed quickly, and further buds were formed in their axils at this point, so that in a few weeks' time several branches appeared to come off from the same level of the main stem. All these, branches or petioles, had swollen basal ends. In the controls the first axillary buds did not develop, and the base of the petiole of the first leaf remained normally thin.

On cutting a section through the swollen base of the petioles of the treated plants all the parenchyma cells were found to be packed with starch grains. Instead of normal rapid stem elongation taking place, food storage and 'tuberization' had been produced by cutting down the length of day.

These short plants produced flowers four days earlier than the tall control plants ; only a few pods were produced by the treated plants ; though they contained fewer seeds, the seeds were remarkably heavy.

On examining the roots, another striking difference was observed between the control plants and all the treated plants (receiving twelve, nine, and six hours' daylight). The treated plants possessed a thick swollen 'tap-root', measuring about ¾ in. in diameter at the top and tapering down to normal root thickness at the bottom, being about 6 ins. in length. When this fusiform root was sectioned, it was seen that secondary growth had taken place. The primary xylem was normal ; a broad band of cambium and newly produced thin-walled cells of a width of as many as eight or nine cells, so rapid was the rate of division evidently, was devoid of starch. The secondary xylem consisted of parenchymatous cells packed with starch grains. Here and there amongst this storage tissue a few lignified elements were distinguishable. Amongst the thin band of secondary phloem formed, parenchyma was also to be found separating the sieve-tubes. Much starch was also stored up in these cells. Owing to the amount of expansion that had taken place, little could be seen of the primary phloem or original cortical tissues.

Briefly, secondary growth had taken place to form more starch storage tissue.

One might have supposed that a plant subjected to shorter days would have been subject to a general carbohydrate starvation, and show symptoms of this. This is far from being the case with this species. Given mild winter conditions there does not seem to be any reason why these short-day plants possessing tuberized roots should not behave as short-lived perennials. Certain varieties of *Phaseolus multiflorus* are known to over-winter in Cornwall.

2. *Chrysanthemum* (var. *Mrs. William Buckingham*). Ref. No. Ds. 32. (Photograph, Plate XVII, Fig. 6).

Small cuttings made in November 1923 were transplanted and subjected to treatment on 9th May 1924. Many well-formed buds appeared on the treated plants by July 26th. These flower-buds opened fully by 6th August 1924. No flower-buds were seen on the control plants until 26th August 1924. The treated plants were more profusely branched than the controls. All the plants were perfectly healthy ; one solitary terminal flower-bud appeared on one control plant on 30th May 1924 ; it never developed, and on growth of the lateral buds taking place it died. The controls flowered fully in November, 2½ months later than the treated plants.

3. *Soya Max. Soy Bean.*

Whilst it has not been possible to carry out similar extensive trials to those of Garner and Allard, smaller trials have yielded some interesting results. Three varieties, whose behaviour under shorter days in America had been studied, were tried. Considering our temperature, they did quite well ; they were started in a cool greenhouse. Plants raised from seed sown 5th May 1924 were subjected to treatment from 31st May. Twenty plants were used in each series ; all the series were watered with a soy bean nodule bacteria solution on 2nd July. The control plants grew much more rapidly than the treated plants, and remained a lighter green colour.

(1) *Mandarin Variety*[1]*—early.* Ref. No. As. 77.

	Ht. in inches 15/7/24.	Flowering Notes.
Healthy control plants	10	Flower-buds 15/8/24
,, 12-hours plants	6	,, ,, 29/7/24
{ 9-hours plants	4	,, ,, 29/7/24 }
{ 6 ,, ,,	3	,, ,, 29/7/24 }

(2) *Peking Variety.* Ref. No. As. 78.

	Ht. in inches 1/8/24.	Flowering Notes.
Healthy control plants	10	No flower-buds 20/8/24
,, 12-hours plants	6	Buds 29/7/24
{ 9-hours plants	3½	,, 29/7/24 }
{ 6 ,, ,,	3	,, 29/7/24 }

[1] Description of types and varieties by Piper and Morse (28).

On 26th August the twelve-hours plants bore small pods, the controls had not yet developed flower-buds; flower-buds were first seen on 29th September on the controls, two months later than the treated plants.

(3) *Biloxi Variety—late.* Ref. No. As. 76.

	Ht. in inches 26/8/24.	Flowering Notes.
Healthy control plants	13	No flower buds 28/9/24
„ 12-hours plants	11	Possessed flower buds 9/8/24
9-hours plants	8	„ „ „ 5/8/24
6 „ „	3	„ „ „ 5/8/24

Controls possessed flower-buds on 7th October 1924—two months later than treated plants.

From these results it is seen that by reducing the period of daylight not only is vegetative growth reduced, but at the same time flowering is accelerated. The later varieties, Peking, and particularly Biloxi, show the effect of shortening the day to a greater extent than the early variety Mandarin. There is no doubt that vegetative growth is related to the length of day.

The results shown in brackets are from plants whose leaves were somewhat injured by toxic vapour from the preservative used on the hut walls and are thus not so trustworthy, yet they do show the same tendency as the results from healthy plants.

Division III. Long-day Plants.

The grass plants subject to treatment were equal propagants made by breaking up a large plant of approximately one year's growth into four equal smaller plants. A week or so after transplanting, the tops of these plants were clipped to about $1\frac{1}{2}$ in. to 2 in. above ground level. All the top growth produced was thus produced whilst the plants were under treatment. Treatment commenced on the 1st of February. The 12-hours plants received all the available light until the days had lengthened to beyond twelve hours. All the plants remained perfectly healthy throughout, being particularly free from disease.

Observations were made upon the time of flowering, the different stages being noted as far as possible. Preliminary to panicle exsertion the tillers become erect; it was found upon examination that erect tillers in treated plants did not necessarily contain, or produce, flower primordia. For this reason the actual appearance of the first panicle emerging from its sheath was regarded as a better time index of flowering than tiller erection. It is realized that the actual day of exsertion of the anthers, and of pollen liberation, depends upon weather conditions, particularly sunshine and temperature. Differences in time here recorded are, however, so great in the majority of

cases that such fluctuations are not of significance.   A fairly reliable estima-
tion can be made of the time of 'zenith flowering', by which is meant the
time when the majority of panicles (clovers : flower-heads) are producing
pollen.   In practically every case the order of reaching the 'flowering zenith'
was that of first panicle emergence.   Experiments were carried out with
these species : early, Sweet Vernal, Foxtail ; medium, Cocksfoot ; and late,
Timothy.   Notes were taken regularly throughout the growing season.

1. *Anthoxanthum   odoratum.*   Sweet-scented   Vernal   Grass.   Ref.   No.
    15 Bs. 1 (3).   (Photograph, Pl. XVII, Fig. 7.)

Treatment.	Date of 1st panicle emergence.	'Zenith flowering.' (Much pollen.)	Panicle height. 30/7/24.	Notes.
Control plants	25/4/24	28/5/24	28 in.	76 panicles produced.
12-hours plants	11/5/24	5/6/24	15 in.	70 panicles.
9 ,, ,,	11/5/24	14/6/24	15 in.	35 ,,
6 ,, ,,	19/5/24	30/6/24	12 in.	Only 12 very poor panicles produced.

A later narrow leaf type gave the following results :

Control plants. (Ref. No. 15 Bs. (1) 1.)	25/4/24	28/5/24	19 in.	40 panicles.
12-hours plants	25/4/24	11/5/24	29 in.	35 ,,
9 ,, ,,	2/5/24	19/5/24	10 in.	18 ,,
6 ,, ,,	19/5/24	—	7 in.	1 poor panicle— never produced pollen.

From the above tables it is seen that by reducing the length of day the
early broad-leaf type was made to flower later.   Not only was flowering
delayed, but the number of panicles produced was reduced.   The panicles
were borne on shorter stalks by those plants receiving less light.

In the case of the narrow-leaf type, which normally flowers later, by
reducing the light to six hours daily, flowering has been practically elimi-
nated.   Vegetative growth was good; a thick tuft of perfectly healthy, long
leaves was produced.

On dissecting the tillers of the plant which had not 'flowered' visibly,
flower primordia, consisting of minute outer glumes enclosing a group of
meristematic cells, were found in some tillers.   No differentiated stamens or
caryopses were produced.   Moreover, the nodes of what should have formed
a long inflorescence stalk remained close together, elongation being pre-
vented.   There is almost a quantitative relationship between the number of
panicles produced and the duration of the light period.

It is interesting to note that the plants have made an attempt to
flower even in the case of the six-hours series.   Further, different pure
lines, as these series were, have reacted differently to these conditions, the

earlier type being able to flower under a six-hours day, whilst the later type failed to produce visible flowers.

2. *Alopecurus pratensis—Meadow Foxtail. Early Hay type* (Ref. No. 277 Bh. 7. Photograph, Pl. XVII, Fig. 8)—gave the following results :

> Controls reached 'flowering zenith' 28/5/24, producing 30 panicles 38 in. high on 31/7/24.
>
> 12-hours plants reached 'flowering zenith' 28/5/24, producing 36 panicles 24 in. high on 31/7/24.
>
> 9-hours plants reached 'flowering zenith' 5/6/24, producing 30 panicles 19 in. high on 31/7/24.
>
> 6-hours plants reached 'flowering zenith' 5/6/24, producing 25 panicles 17 in. high on 31/7/24.

Whilst the *late Pasture type* (Photograph, Pl. XVII, Fig. 9. Ref. No. 278 Bh. 21, confirmed by 278 Bh. 4) results can be summarized for comparison thus :

> Control reached 'zenith flowering' 6/6/24, producing 32 panicles 33 in. high on 31/7/24.
>
> 12-hours plants reached 'zenith flowering' 6/6/24, producing 20 panicles 25 in. high on 31/7/24.
>
> 9-hours plants reached 'zenith flowering' 30/6/24, producing 17 panicles 17 in. high on 31/7/24.
>
> 6-hours plants reached 'zenith flowering' 31/7/24, producing 5[1] very poor panicles 13 in. high on 31/7/24.

Again, flowering has been retarded and the later flowering pure line of this early grass has suffered more from the light reduction than the earlier flowering type.

3. *Dactylis glomerata—Cocksfoot.*

The results obtained from a range of types of Cocksfoot selected for 'earliness' and 'lateness' will now be considered. These types are pure lines. Two series of each type were subjected to treatment ; the results of one are presented.

'*French Hay*'. An early type.[2] Ref. No. 812 Bc. (1) 24 (results confirmed by behaviour of 812 Bc. (1) 17). (Photograph, Pl. XVII, Fig. 10).

Treatment.	Date of 1st panicle emerging.	'Zenith flowering' and pollen.	Panicle height and notes on 31/7/24.
Control plants	19/5/24	5/6/24	34 in.—ripe seed.
12-hours plants	6/6/24	16/6/24	15 in.—ripening seed.
9 ,, ,, 6 ,, ,,	Remained in the winter habit, good leafy growth and tillers, but no signs of panicles at all on 26/10/24.		

[1] Very little mature pollen was produced by these panicles.
[2] Description of types by R. G. Stapledon (30).

'*Danish Open*' *type*.   Ref. No. 833 Bc. (1) 28, confirmed by behaviour of 833 Bc. (1) 4.

Treatment.	Date of 1st panicle emerging.	'*Zenith flowering*' and pollen.	Panicle Height and Notes on 31/7/24.
Control plants	23/5/24	18/6/24	33 in.—ripe seed.
12-hours plants	28/5/24	27/6/24	10 in.—ripening seed.
9 ,, ,,	No signs of flowering, good leafy growth on 26/10/24.		5 in.
6 ,, ,,	No panicles all the season.		

' *Late Indigenous*' *Type*.   Ref. No. 828 Bc. 13, confirmed by 828 Bc. 22.   (Photograph, Pl. XVIII, Fig. 11.)

Control plants	25/5/24	14/6/24	29 in.—ripe seed.
12-hours plants	27/6/24	25/7/24	9 in. panicles just closing again.
9 ,, ,,	Plants with good leafy growth, no signs of panicles 26/10/24.		
6 ,, ,,	No inflorescences all the season.		

' *Tussocks*' *Type*.[1]   A very late type.   Ref. No. 914 Bc. (1) 6, confirmed by 914 Bc. (1) 5.   (Photographs, Pl. XVIII, Figs. 12 and 13.)

Control plants	29/5/24	25/6/24	27 in., ripening.
12-hours plants	30/6/24	29/7/24	9 in., flowering just ceasing.
9 ,, ,,	As yet in winter habit 26/10/24.		
6 ,, ,,	No inflorescence all the season.		

All these plants were quite healthy.   The 12-hours plants of this type, instead of ripening normally, went back to the vegetative state of growth ; the glumes elongated, and by September had the appearance of small leaves.   The whole inflorescence resembled the type found in viviparous Fescues.   The plants subjected to the short days produced good leafy growth and new tillers slowly; the panicles produced under even twelve hours' illumination were decidedly poorer than those of the control plants.   On dissection of the tillers of the plants subjected to nine and six hours no signs of panicle primordia were seen.

4. *Lolium perenne—Perennial Rye Grass.*

A small trial with a very early and a very late type of this species was carried out with the following results :

Early Type.   Ba. 200.   (This parent plant broke up into somewhat unequal parts.)

Control plant reached 'flowering zenith' 12/6/24, producing about 14 spikes.

12-hours plant reached 'flowering zenith' 12/6/24, producing about 14 spikes.

9-hours plant reached 'flowering zenith' 28/6/24, producing about 11 spikes.

6-hours plant reached 'flowering zenith' 28/6/24, producing about 2 spikes.

[1] Description by R. G. Stapledon (31).

Late Type.

Control plant started flowering 27/6/24, reaching 'flowering zenith'
   29/7/24.

12-hours ⎫ plants produced good leafy growth—a compact small green
 9-hours ⎬ tuft—with no signs of spikes 28/9/24. No inflorescences
 6-hours ⎭ produced all the season.

There is thus seen to be a striking difference between the behaviour of
the early and late type under the shorter day conditions. The early type
can flower under six hours' daylight, the late cannot produce flowers even
when it receives twelve hours' daylight.

5. Phleum pratense—Timothy.

Timothy, being normally the latest of the grasses to flower, was
expected to give rather different results than the early grasses (Foxtail
and Sweet Vernal) or than the medium grasses (e. g. Cocksfoot). It was
thought that ' Late ' Timothy might even be a short-day plant.

Early Type. Ref. No. Bd. 92. (Photograph, Pl. XVIII, Fig. 14.)

Control plant's first panicle emerged on 5 June 1924; ' flowering zenith '
was reached 31 July 1924—24 panicles about 36 in. high; ripe on 11 Sept.
1924. The 12-hours plant possessed erect swollen shoots 31 July 1924, as
if panicles were about to emerge. On 22 Aug. 1924 the appearance of
this plant had not altered; on dissecting it, in some tillers flower primordia
were found upon a yellow rachis enclosed by a yellow leaf, the outermost
leaf being normal. The young flowers had been checked in their develop-
ment and were dying off; they consisted of well-formed outer glumes
enclosing a small group of meristematic tissue: no palae, caryopsis, or
stamens had been differentiated.

The 9-hours plant closely resembled the 12-hours plant from 31 July
1924. The 6-hours plant was also similar to the 12-hours plant, having
tillers swollen up as if panicles were about to emerge—some of these tillers
also contained arrested young flowers, 22 Aug. 1924.

Late Type. Ref. No. Bd. 48. (Photograph, Pl. XVIII, Fig. 15.)

Control plant flowered normally, reaching ' flowering zenith ' 11 Aug.
1924. The 12-hours plant has produced leaves and new tillers, but has
remained in the winter habit, more or less flat or prostrate, 22 Aug. 1924.

The 9-hours plant resembled very closely the 12-hours plant. The
6-hours plant from the beginning of May appeared as if it would flower
earlier than the 12-hours and 9-hours plants and as early as the control
plant. On 21 June it possessed upright tillers as if about to flower; having
reached this stage it never progressed farther, and no panicles had emerged
by 28 Aug. 1924.

The early type of Timothy behaved as a long-day plant, kept from

full flowering by shortening the day, though able to form primordia and upright tillers.

Further investigation of this late type is required. Indications that the late flowering type of Timothy under the six-hours day will actually go through the initial stages of flowering, whilst a plant of an earlier flowering species (Cocksfoot) remained in the winter habit when placed under a six-hours day, have been obtained. (It should be noted, however, that in the case of the Timothy the basal 'bulbs' of the tillers were not removed when the plants were clipped, so that some food supply would be available.) From such considerations it is seen that varieties, strains, or species that are relatively earlier or later than one another under full natural illumination will not necessarily flower in the same relative order under shorter light periods.

One of the most interesting results obtained from the grasses is that the majority of late strains show a comparatively higher and possibly narrower range of length of day in which they can flower, whereas the earlier types can produce flowers in a lower range of length of day. The later types are indigenous and are of particular pasture value, because of their persistency, and are characterized by giving prolonged vegetative growth and a high ratio of leaf to stem. For example, Late Indigenous Cocksfoot does not flower under days of twelve hours with usual vigour, whereas French Hay Cocksfoot does.

6. *Avena sativa—Oats.*

Pure line seed was sown on the 1st February in large Doulton pots placed on the trucks, so that from the very first the plants were subject to treatment. Unfortunately the plants which were in the dark for eighteen hours and fifteen hours suffered from the attack of slugs, so that it was necessary to reduce the number of replications and to transplant some of the seedlings. Two pots of three plants each of 'Grey Winter', and one pot (three plants) of the following spring varieties, 'Orion', 'Record', and Ceirch-du-bach', remained in each series throughout most of the season.

Grey Winter. Ref. No. 843/3 Cc.

	Av. No. tillers 5/6/24.	Length of longest leaf.	Notes. 27/6/24.	Notes. 29/7/24. Ht. in inches.	Notes. 11/8/24.[g]
Control plants	12	14 in.	Swollen high up, panicles about to burst out	Full flowering	Three panicles out. Now ripening.
12-hours plants	16	14 in.	Swollen at base only	About 16 in.— as on 27/6/24	No flowers. No panicles have emerged.
9 ,, ,,	18	10 in.	Slightly swollen at base	About 12 in. tall	
6 ,, ,,	9	10 in.	No swelling at base	About 11 in. tall	

The 12-hours plants produced panicles in October, three months later

than the controls. These panicles were borne on very short stems. Tillering was prolonged slightly by reducing the length of day. The 9- and 6-hours plants appeared quite green in October ; possibly they may over-winter and function as short-lived perennials.

Orion. Normally one of the earliest varieties. Ref. No. 2477/1 Cc.

	21/5/24.	12/6/24.	27/6/24.	11/8/24.
Control plants	2 tillers, longest leaf 12 in.	About to burst into panicle	1st panicles well out, 40 in. tall	1st and 2nd panicles fully ripe, glumes yellow ; 2 tillers well developed.
12-hours plants	4–5 tillers, longest leaf 10 in.	Swelling up near base	Swollen higher up, 32 in. tall	1st panicle out; 2nd panicle coming out ; 2 tillers well developed.
9 ,, ,,	2 tillers, longest leaf 10 in.	Longest tiller swelling up at base	23 in. tall	1st panicle bursting through ; 2 tillers well developed.
6 ,, ,,	1 small tiller	1 small tiller, very poor plants. Slug attacked also.	Dead	

Decreasing the length of day has retarded flowering at all the stages ; the treated plants have been several weeks behind the controls.

Record. Normally a medium ripening variety. Ref. No. 1642/2 Cc.

	27/6/24.	29/7/24.	11/8/24.	28/8/24.
Control plants	5 tillers, 20 in. tall	In full panicle. Very tall (32 in.,	Glumes and straw green	Ripe grain. 4 tillers well developed.
12-hours plants	7 ,, 18 in. ,,	About 20 in. high, swelling up	Swollen, but no panicles out	3 tillers well developed.
9 ,, ,,	5 ,, 14 in. ,,	18 in. tall	No panicles	— 1
6 ,, ,,	4 ,, 11 in. ,,	About 12 in. tall	,, ,,	Tillers well developed.[1]

Decreasing the length of day has prevented flowering and prolonged vegetative growth. By producing new tillers the treated plants have entered upon a second year's growth (6th March 1925).

Ceirch-du-bach. A late-ripening spring variety. Ref. No. 952/4 Cc. (Photographs, Pl. XVIII, Figs. 16 and 17.)

	5/6/24.	27/6/24.	15/7/24.
Control plants	7 tillers, longest leaf 16 in.	7 tillers, swollen slightly at base	Panicles out. Very tall (38 in.).
12-hours plants	10 tillers, longest leaf 13 in.	10 tillers, no swelling at base	No signs of swelling 29/7/24, about 16 in. tall.
9 ,, ,,	6 tillers, longest leaf 11 in.	8 tillers	About 14 in. tall.
6 ,, ,,	1 tiller, leaf 6 in., very poor	6 tillers about 10 in. high	About 12 in. tall.

No visible panicles produced.

	11/8/24.
Control plants	Glumes green, straw slightly yellow.
12-hours plants	A slight swelling at base. Poor panicles produced in October.

The late variety has been made to tiller more by prolonging vegetative growth. Flowering has been greatly retarded.

The results obtained from oats are in general agreement with those obtained from other grasses, and may be thus summarized:

(1) Shortening the day retards flowering and makes the early varieties late, and prevents the later varieties from flowering.

(2) It tends to make the tillering period longer, so that more tillers are produced. The space (size of pot) factor has undoubtedly had an effect also, tending to minimize the length of day effect.

(3) Although unable to flower normally, the plants subjected to the shorter days have left the rosette tillering stage and shot up into an erect habit, as if about to flower; only a few of the other grasses (e. g. Timothy, early type) did this.

(4) On examination, the roots of the grasses and oats subjected to short days appeared normal.

17. Trifolium pratense—Red Clover.

A range of types of Red Clover was subjected to treatment from 1st February. The plants were approximately fourteen months old when treatment started. Previous to transplanting they had been clipped to ground level. Two 'matched' plants of Broad Red, an early East Anglian type; of American Medium Red, a medium flowering type; and of Montgomery, a particularly late flowering type; were placed in each series. Besides this a further pair of equal propagants (in this case = cuttings) from the same parent plant of late flowering Montgomery type were subjected to each treatment as a check upon the matched selection.

' *English Broad Red.* An early-flowering type. Ref. No. Aa. 505. (Photograph, Pl. XVIII, Fig. 18.)

Control plants produced erect stems by 28th April 1924, flower-heads were formed by the 12th May, and by the 6th June the ' flowering zenith ' was reached, when much pollen was produced by many large flowers in many heads.

12-hours plants were erect on the 28th April, but did not produce well-formed flower-heads until 27th June (about three weeks later than control). The ' flowering zenith' was reached on 26th July, nearly seven weeks later than the controls; there were not so many flower-heads or as many flowers per head as in the controls.

9-hours plants took longer to become erect than the 12-hours plants, but flowered very nearly at the same time, producing so few flowers open at once that a very accurate estimate of the 'flowering zenith' was not possible; started to flower 6th July, 'flowering zenith' reached about 31st July. These plants approached the ever-blossoming condition, flowers still being formed in November.

6-hours plants have remained in the winter habit, producing many new small leaves. No stem elongation has taken place, the plants were prostrate in September, and would appear to have missed a season's flowering.

American medium type (Ref. No. Aa. 510. Photograph, Pl. XVIII, Fig. 19) gave the following results:

> Control plants—erect 7/5/24. Appearance of first coloured flower-heads 13/6/24. 'Flowering zenith' 18/6/24.
>
> 12-hours plants—erect 28/5/24. Appearance of first coloured flower-heads 15/7/24. 'Flowering zenith' 5/8/24.
>
> 9-hours plants—some erect branching 27/6/24. Appearance of first coloured flower-heads 29/7/24, few flowering heads still being formed 11/8/24, but plants not erect. These plants approached the ever-blossoming habit.
>
> 6-hours plants have remained prostrate and entirely healthy, forming many new leaves.

Montgomery.[1] A very late flowering type. Ref. No. Aa. 485. (Photographs, Pl. XVIII, Figs. 20 and 21.)

> Control plants—erect 21/5/24, flowering zenith 29/7/24, very many flowers.
>
> 12-hours plants ⎫ have remained as dense prostrate 'mats' with crowded
> 9-hours   „   ⎬ leaves. They have missed a season's flower pro-
> 6-hours   „   ⎭ duction.

This result was confirmed by the behaviour of the equal propagants of Montgomery late flowering variety (Ref. No. Aa. 616, propagants). Control plants became very tall and erect, producing an abundance of flower-heads reaching the 'flowering zenith' on 29 July 1924. They contrasted markedly with the leafy flat growth of the 12-, 9-, and 6-hours plants. The roots of the late Montgomery type (Aa. 485) were examined, and it was found that the 12-hours plants possessed many more thick roots than did the control plants. The 9-hours plants possessed slightly thicker roots than the control, whilst the 6-hours plants showed roots very much like the control in thickness. It would appear that some food storage in the roots had taken place in plants subjected to a 12-hours day and prevented from becoming erect and branched (ready to flower).

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[1] Description of types by R. D. Williams (35).

8. *Raphanus sativus—Radish,* 'Scarlet Globe', Sutton's.   Ref. No. Ds. 25.
   (Photograph, Pl. XIX, Fig. 22.)

Seed was sown in boxes on 1 Feb. 1924; after establishment the plants were thinned down on 22 April 1924.   From the first all these plants were subjected to the four different periods of illumination.   The control plants produced a rosette of leaves, as did the treated plants, but stem elongation took place more rapidly in the former.

Control plants approximately 3   in. high on 21/5/24.
12-hours plants   „   2 in.   „   „
    9-hours   „   „ $1\frac{1}{2}$ in.   „   „
    6-hours   „   „ $\frac{1}{2}$ in.   „   „

Flower-buds appeared quickly, so that on 28th May the controls were 4 in. high with well-formed buds.

12-hours plants were 3 in. high with well-formed flower-buds.
    9-hours   „   2 in.   „   no visible flower-buds.
    6-hours   „   1 in.   „   „   „   merely a rosette.

The dates of flowering are shown thus:

Control plants: first flowers 1/6/24, 8 in. tall; 5/6/24, all flowering,
        10 in. high.
12-hours plants: first flowers 3/6/24, 4 in. tall; 6/6/24, all flowering,
        4 in. high.
9-hours plants: first flowers 18/6/24, 4 in. tall; 27/6/24, all flowering,
        5 in. high.
6-hours plants: first flowers 27/6/24, 5 in. tall; 3/7/24, all flowering,
        5 in. high.

Ripening of the fruits of the control, 9-hours, and 6-hours took place in a similar order.

The 12-hours plants were transferred to the 6-hours section, with the following results:

(1) Large buds formed in long (12-hours) days opened, though slowly, in short (6-hours) days.

(2) Many of the plants died off.

(3) Ripening of fruit was greatly delayed in other plants.

(4) A plant which had produced flowers, and had been fertilized and bore small fruits, produced by 28 August 1924 a rosette of leaves amongst the small fruits.   Stem elongation was checked and vegetative growth was resumed slowly by this plant.

(5) None of the plants produced enlarged roots of any size.

Division IV. Intermediate Types.

Digitalis purpurea. Foxglove. Ref. No. Ds. 27. (Photographs, Pl. XIX, Figs. 23 and 24.)

A small trial was carried out with this species to test the effect of length of day upon rosette plants. From the locality equal rosettes were transplanted and subjected to treatment, starting 1st Feb. The 12-hours and control plants started to elongate and produce a flowering shoot before the other treated plants, 9- and 6-hours. The growth of these inflorescence stalks can be shown best thus:

	Date.	Height of Inflorescence Stalks—Foxglove.				
	28/4/24.	17/5/24	23/5/24	31/5/24	5/6/24	31/7/24
Control plants	Slightly raised	8 in.	15 in.	19 in·	26 in.	39 in.
12 hours plants	,, ,,	11 in.	21 in.	31 in·	38 in.	50 in.
9 ,, ,,	Normal winter rosette	6 in.	11 in.	18 in.	25 in.	34 in.
6 ,, ,,	Normal winter rosette	4 in.	8 in.	10 in.	14 in.	20 in.

It is to be noticed that the greatest growth in height was produced by an intermediate length of day. Despite this great difference in growth of the inflorescence stalks, there was comparatively little difference between the time of flowering of the plants.

	Flower-buds well developed.		Petals opened.	Pollen production.
Control plants	Well-formed flower-buds 23/5/24		Coloured (basal) flowers 6/6/24	Opened and pollen 11/6/24.
12 hours plants	·,	,,	15/5/24	,,	Opened and pollen 10/6/24.
9 ,, ,,	,,	,,	29/5/24	,,	Opened and pollen 11/6/24.
6 ,, ,,	,,	,,	,,	,,	Opened and pollen 11/6/24.

As the flowering axis had not elongated so much in the 9- and 6-hours' plants, the flowers were very close together near the top of the inflorescence, and some were not fertilized and died off unnaturally. ·

Ripening of the fruits produced proceeded normally in all cases. In September it was noticed that the plants subjected to 9 and 6 hours had formed new small leaves at the base of the inflorescence—no such leaves were to be seen on the control and 12-hours plants. After flowering, vegetative growth was resumed to a slight extent by the plants subjected to short days.

On examination, the roots of all the plants appeared normal (29 August 1924).

Digitalis purpurea has been placed in this intermediate division, for it serves as a link between 'short-day' plants which respond to shortened days by flowering earlier, though short in stature, and 'long-day' plants which under shorter days do not elongate normally, and flowering is retarded. In the case of the intermediate plants flowering has not been

accelerated or retarded, though growth has been influenced. A similar case has been described by Garner and Allard (9),[1] who found that the length of day greatly affected the stature of *Helianthus annuus* (Sunflower), though the time of flowering was not materially altered.

Division V.   Results with Types that did not flower.

1. *Helianthus tuberosus (Jerusalem Artichoke).* (Dickson's.)   Ref. No. Ds. 24.   (Photographs, Pl. XIX, Figs. 25, 26, and 27.)

Tubers planted 1 Feb. 1924 were up and forming young leaves by 7 April 1924; from planting these were subjected to treatment. The control plants grew in height very much more rapidly than did the treated plants, which showed a gradation in height varying with the length of day.

Heights of Artichoke Stems to Apical Bud.

	21/5/24.	28/5/24.	5/6/24.	31/7/24.
Control plants	7 in.	14 in.	22 in.	40 in.
12-hours ,,	6 in.	7 in.	10 in.	26 in.
9 ,, ,,	5 in.	7 in.	9 in.	24 in.
6 ,, .,	4 in.	6 in.	7 in.	24 in.

All the plants were healthy, those subjected to shorter days developed their leaves normally, and were in consequence of their shortness particularly 'leafy' as compared with the tall controls. Towards the end of the summer the control, 12-, and 9-hours plants showed signs of autumn colour change in the leaf, the 6-hours plants possessed perfectly green leaves. The plants were dug up to examine the tuber formation on 28 Aug. 1924. The control plants had produced very small, poor tubers. A large number of medium size, regular-shaped tubers were produced by the 12-hours plants. An equally good crop was produced by the 9-hours plants. The 6-hours plants did not produce so many tubers, but those produced were large and irregular. In the shorter days 'tuberization' had been intense, the controls often possessing long, swollen, underground stems.

Weight of Tubers produced (eight Tubers[2] originally sown).

Control plants	107 grm.	Small and few tubers.
12-hours ,,	808 ,,	Medium size, regular, numerous.
9 ,, ,,	803 ,,	,, size, few, ,, ,,
6 ,, ,,	457 ,,	Large size, few, irregular.

There can be little doubt that shortening the length of day favours tuberization and reduces elongation in this species.

2. *Brassica oleracea. Cabbage.* (Gibbs's White 'Prize-taker' spring Cabbage.)   Ref. No. Ds. 26.   (Photograph, Pl. XIX, Fig. 28.)

A small trial with this species was carried out. Seedlings (sown 3 Aug.

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[1] Journ. Agric. Research, vol. xxvii, No. 3, 1924, p. 145.
[2] Total weight of 8 tubers as nearly equal as possible.

1923) consisting of a four-leaf rosette were transferred to treatment 8 Feb. 1924—four plants per treatment. Growth was slow, but it was seen that the plants subjected to shorter days were growing faster, producing more leaf and stalk, so that later a crown of new leaves above the ground was formed, whilst the control rosette remained at soil level.

	21/5/24.	22/8/24.
Control plants	3 in. tall, little or no stalk produced	About $3\frac{1}{2}$ in. tall. Smaller leaves than treated plants, less stalk.
12-hours plants	3–4 in. tall	About $5\frac{1}{2}$ in. tall. More stalk produced and larger leaves.
9 ,, ,,	$4\frac{1}{2}$ in. tall	About 6 in. tall.
6 ,, ,,	5 in. tall	., 8 in. .,

Shortening the day has increased the leaf formation and accelerated stem elongation of this variety. Such a result has not been obtained with other species.

3. *Solanum tuberosum. Potato.* 'King Edward', Ref. No. Ds. 28; 'Sharpe's Express', Ref. No. Ds. 29. Grown from tubers planted 22 April 1924.

Although the leaves of these plants were not healthy at the latter part of the growing season, yet the plants serve to bring out a few general points. The control and treated plants grew equally at first. Translocation from the parent tuber to the young plant had not been affected. No flowers were produced. The results obtained with 'King Edward' and 'Sharpe's Express' bear out those obtained by Garner and Allard with 'Irish Potato'.

Mass of tubers 'King Edward', Ref. No. Ds. 28.		'Sharpe's Express', Ds. 29.
Control plants	65 grm.	148 grm.
12-hours plants	90 ,.	120 ,,
9 ,. ,.	29 .,	51 ,,
6 ., ,,	12 .,	18 ,.

Dug up on 31st July.

In the former case there is an optimal length of day favouring tuber formation. (In the latter an optimum may exist.) The shortest day plants show the reduction of carbohydrate due to lack of photosynthesis. Similar results with Irish Potato, obtained by Garner and Allard[1] (10), are quoted below:

18 hours' light—vegetative growth, flower-buds, no tubers.

14–15 hours' light—flowers and moderate tuber formation.

13 hours' light—increased 'tuberization'.

10 hours' light—very intense 'tuberization', compared with 13 hours for same 'tops', seven times as much weight of tuber.

5 hours' light—very much less 'tuberization'.

The optimum for tuber formation would appear to be a varietal character.

[1] Journ of Agric. Research, vol. xxiii, No. 11, p. 897, 1923.

GENERAL DISCUSSION AND CONCLUSIONS.

The results of the experiments under review can only be adequately dealt with when consideration is also given to work undertaken by other investigators on various aspects of the phenomena of flowering and fruiting. In the discussion which follows, therefore, an attempt is made to draw certain conclusions or inferences which appear to be justified from the results viewed in the light of current literature on related subjects.

I. *The American and Welsh Results—Comparisons.*

At the outset it can be stated that the results obtained from this first season's trial, by reducing the length of day, are in close agreement with those obtained by Garner and Allard.

By reducing the length of day, plants that normally flower in autumn or after midsummer are brought into flower earlier; plants which normally flower in the long days of early summer are, on the other hand, prevented from flowering.   (Differences in growth will be considered later.)

From the point of view of these experiments where the controls were out of doors, the differences between the American (Washington) and Welsh (Aberystwyth) conditions that are most striking are these:

1. The northern latitude of Aberystwyth ensures a wider range of seasonal variation in the length of day.

2. Generally the temperature throughout the summer is much lower in this country and the rainfall higher.

Despite these differences, the type of response produced by this shortening-the-day-treatment has been of the same kind for the species under investigation at both places.   The results obtained with *Soya max, Trifolium pratense, Helianthus tuberosus, Raphanus sativus, Phaseolus multiflorus*, for example, are in close agreement.

The results obtained with Soy Beans in this country and at Washington may be considered as an illustration of the type of difference obtained. The time taken by plants of the variety 'Biloxi', when subjected to approximately the same length of day, to reach the flowering stage varies, thus:

Washington.[1]   BILOXI SOY BEANS sown 8 May 1918, subject to seven hours' light daily, flowered after twenty-five days' treatment on 15 June 1918, average height 11 in.

Aberystwyth.   BILOXI SOY BEANS sown 8 May 1924, subject to six hours' light daily, produced flower-buds 5 Aug. 1924, after eight weeks' treatment, height 4 in.

The warmer conditions at Washington have hastened growth and flowering; generally all the results which indicate luxuriance of growth (as

[1] Journ. Agric. Research, vol. xviii, p. 562, 1920.

height) are of a smaller order of magnitude in this country. From the point of view of the length of day, it is very interesting to compare the results of the controls.

Washington.[1] MANDARIN SOY BEANS planted 8 May 1918 flowered on 15 June 1918. Average height, 19 in.; length of day when flowering, fifteen hours (approximately).

Aberystwyth. MANDARIN SOY BEANS planted 8 May 1924 produced well-formed flower-buds on 15 Aug. 1924. Length of day when flowering, approximately fifteen hours ; average height, $11\frac{1}{2}$ in.

It is seen that the length of day when this variety flowers is equal in the two countries. The varieties Peking and Biloxi have also been found to come into flower later in this country than under the American conditions. In this connexion it is to be noted that not until October do our days shorten down to a length comparable with those of Washington at the beginning of September, when the control Biloxi Soy Beans flowered. Control Biloxi plants flowered on 9 Oct. 1924 in this country.

II. *The Nature of the Stimulus producing the above Results Length of Day. 'Photoperiodism.'*

It is apparent that by running the plants into the darkened hut the period of active photo-reduction is curtailed, and that the rate of transpiration decreases upon the closing of the stoma.

Evidence has been collected by Garner and Allard (7) to show that the effects produced cannot be entirely explained by a consideration of photosynthesis alone. A brief review of some of this evidence may not be out of place at this point.

It has been demonstrated that darkening Soy Beans, and other species, for a period of hours in the middle of the day does not accelerate flower production. An interrupted period of darkness does not produce the same effect as a continuous long night. Emphasis is thus laid upon the importance of the duration of the light (and dark) period.

A series of experiments were carried out in which the light period was prolonged by very weak artificial electric light (five foot-candles). This weak light lengthened the days so that 'short-day plants' were prevented from flowering and vegetative elongation continued to great heights (e. g. *Cosmos*). Under these conditions 'long-day plants' were brought into flower earlier. It is doubtful whether such a weak light would cause an appreciable increase of photosynthetic activity. This series of experiments lays further stress upon the importance of the duration of the light period.

The most substantial confirmation of the view that it is the length of day that is of importance lies of course in the consideration of seasonal

[1] Ibid., p. 574, 1920.

variation.   In the tropics [1] there is no marked seasonal difference in length of day and no general seasonal regularity of flowering.   Outside the tropics the regularity with which species blossom within a narrow margin of time year after year is such a commonplace phenomenon that it is apt to be over-looked. Whilst temperature, rainfall, and other weather conditions do cause fluctuations in the time of blossom production, spring-flowering types remain spring-flowering types, and those that flower in the long days of early summer are not made to flower in late autumn by such weather fluctuations.

A series of rows of Soy Beans sown at different dates in the open came to maturity at the same time, when the natural daylight period was reduced sufficiently.   These series showed great difference in vegetative growth, those which had been sown early being generally taller and larger plants.

Because of the above evidence the term 'photoperiodism' has been suggested by Garner and Allard 'to designate the response of the organism to relative length of day and night'.

'Length of day' must be a complex factor including such factors as intensity of light, duration of light, reaction of the plant by chlorophyll production, the effect of light upon transpiration and hydration of the tissues—all of which may be controlled and varied by 'length of day' itself. Having considered the operation of these units which are found in 'length of day' it remains to see how far the response produced can be explained. If such considerations fail to explain the response, then it would appear that there must be a 'photoperiodic' factor—that the actual *length* of day (and of night) itself is operative.

III. *Photosynthetic Considerations.   Length of Day and Photo-reduction. Light Intensity.*

In the experiments described in this paper it will be noticed that, as far as could be conveniently arranged, plants subject to shorter days were out in the open during the period of maximum light intensity.   There can be no doubt, however, that the 'intensity × time' measurement of the light received by these plants falls far short of that of the controls, which were out all day.

Considering intensity alone, it is surprising to find that some treated plants (*Phaseolus multiflorus*) possess so much starch that they seem to suffer from an excess rather than a lack of the ultimate product of photosynthesis.

In order to elucidate the effect of light intensity Garner and Allard conducted a series of shading experiments.   By employing a number of differently woven fabrics the light intensity was reduced to 25 per cent. of

[1] McClelland (24) has, however, shown that even small alterations in length of day, as at Porto Rico, control flowering of some species, e.g. *Tephrosia candida.*

the normal. By this treatment Soy Bean flowering was not accelerated, whereas a slight alteration of the length of day produced a marked response. In these experiments, however, it is not certain that assimilation was decreased, for the chlorophyll may have been active at a low light intensity and a reduction of the light may have increased the leaf surface available. It is just possible that the available light energy was utilized in different proportions from the normal manner. For this reason such shading experiments themselves cannot be considered of much value in support of the theory that length of day is of more importance than light intensity in explaining the results obtained by cutting down the duration of the light period.

Chlorophyll Content.

Throughout these experiments no signs of etiolation have been observed. Plants subjected to a period of eighteen hours' darkness show characteristics entirely different from those associated with etiolation. There must be a limit to which the length of day can be reduced before etiolation sets in ; below this limit of daily illumination little or no pigment is formed, exposure to a length of day of six hours results in increased pigmentation. The leaves of Soy Beans subjected to six hours' (Cardiganshire results) and five hours' light (Washington results) are a darker green than the control leaves. To this extent these plants show partial compensation for the reduced light.

Length of Day and Condensation Processes.

It has been pointed out that the length of day may operate by affecting the ' light intensity and time ' product and so controlling the photoreduction reaction; the operation of the same complex factor (i. e. length of day) upon the later stages of carbon assimilation must be mentioned also. Plants subjected to a prolonged night have a longer time in which to carry out condensation reactions; for this reason they may be able to remove the end-products of the initial reactions completely. Having done so, the plant starts the next day with a mechanism unhampered by the presence of its own products. Thus it may be that for some short time the rate of photosynthesis would be high.

From such considerations it is seen that the effect of length of day upon the manufacture of carbohydrate is not only a matter of light reduction, the plant is in some small measure probably able to compensate itself for the loss of longer days. At any rate, surprisingly large quantities of carbohydrates are found in plants subjected to only nine and six hours' daylight.

IV. *Nitrogen Salt and Water Relations.*

Throughout the experiments an abundant supply of water was available. An adequate supply of mineral food existed in the soil. Reduced transpiration might tend to reduce the nitrogen available and so limit leafy growth. The plants subjected to shorter days as a general rule did not appear to suffer from any mineral starvation, often they were comparatively 'leafy'. The differences brought about by altering the length of day by a few hours only, in the degree of hydration of the tissues and of the amount of nitrogen conveyed upwards, must be small. It is hoped that a series of experiments to test the effect of nitrogenous salts when supplied in large quantities to plants subject to various lengths of day may be carried out next season. The difficulty of judging the results of such experiments where nitrogen and water are varied lies in the fact that accurate data cannot be easily obtained of the amount of nitrogen that is actually utilized at any given time by the plant when soil cultures are employed.

V. *Utilization of Food Products. Elongation and Tuberization.*

The following figures, taken from the data given in the earlier part of this paper, show clearly that by shortening the length of day growth in height is limited:

	Biloxi Soy Beans, 3½ *months old.*	*Phaseolus multiflorus,* 3 *months old.*
Control plants	13 in. to apical bud	42 in. ht., twining stems.
12-hours plants	11 in. ,,	10 in. erect and not twining
9 ,, ,,	8 in. ,,	9 in. ,, ,, ,,
6 ,, ,,	3 in. ,,	9 in. ,, ,, ,,

In the case of *Phaseolus multiflorus*, 'tuberization' accompanied reduction in height. The above examples are from short-day plants. The height of grass panicles can be taken as an example of the reduction in height of long-day plants.

	Height attained by *Cocksfoot panicles,* *French Hay Type.*	*Height on* 31/7/24. *Meadow Foxtail,* *Early Type.*
Control plants	34 in. (ripe seed)	38 in.
12-hours plants	17 in. ,,	24 in.
9 ,, ,,	No panicles	19 in.
6 ,, ,,	,,	17 in.

The effect of reducing the length of day upon growth of a non-flowering type, e. g. Artichoke, shows that this phenomenon is not confined to either of the above classes. The Artichoke results fall into a series; at one end of this series long daylight and great elongation are found, at the other short days and intense 'tuberization'. The series are as follows:

	Helianthus tuberosus. *Height to Apical Bud.*		*Tubers produced.*
	4 *months.*	6 *months.*	
Control plants	22 in.	42 in.	107 grm. small tubers.
12-hours plants	10 in.	26 in.	808 „ medium size,
9 „ „	9 in.	24 in.	803 „ regular shape, numerous.
6 „ „	7 in.	24 in.	457 „ larger, fewer.

These examples show how cutting down the length of day regulates the use to which the food manufactured is put.

The results of other workers, particularly those of Garner and Allard, are in close agreement with those above.

In a series of experiments in which the period of daily illumination was prolonged by the use of electric light, Garner and Allard noted that many species grew to a great height. *Cosmos*, for example, grew to 15 ft., whilst remaining in the vegetative state, controls subjected to ordinary daylight remained short and flowered at $1\frac{1}{2}$ ft. In extreme cases (*Poinsettia* and *Cosmos*) upper stem elongation under long-day conditions took place at the expense of the lower leaves, which were shed presumably owing to a lack of food from the roots. As a result of the evidence they collected, these workers state that for each species an optimum light period for maximum elongation exists.

The work of Adams (2) with electric light also shows that prolonged light gives greater growth in height in a further number of species. Adams worked with a wide range of light exposures. His results with Tomato, Soy Bean, Hemp, and Buckwheat indicate ' that increased light-duration beyond a definite maximum produces little if any additional growth'. On the other hand, exceedingly short periods of light checked growth during the earliest seedling stages when compared to the growth of seedlings in complete darkness.

Although short days limit growth and cause tuberization, the length of day cannot be reduced to extreme limits without etiolation taking place. There must be a critical point beyond which reduction of the light period produces etiolation with all its associated features. It may be that the shortest periods of daylight to which Adams subjected his plants fell below the critical point at which some features usually associated with etiolation set it.

Discussing his results, which clearly show that plants receiving daylight for three or five hours daily start by being taller than plants receiving twelve or fifteen hours' light, but are shorter at a later stage than those subjected to long days, Adams (2) states that ' it follows, therefore, that where plants are equally supplied with reserve food material growth in length of an axial organ takes place more rapidly in darkness or in diminished light'. ' In the end, however, those plants exposed longest to the action of light, being able to accumulate a large supply of reserve material for tissue formation by photosynthesis, ultimately attain the greatest height.'

When one considers the features of tuberization, previously described by Garner and Allard (10) and confirmed by the results presented in this paper, where there *is* an exceedingly large supply of reserve material accumulated by photosynthetic activity and yet no growth in height, it is apparent that the effect of the complex factor 'length of day'—the photoperiodic response—cannot be explained by photosynthesis alone. The utilization of the products produced must be considered.

For elongation to take place in healthy tissues a ready supply of sugar and an abundant supply of water are necessary. A copious supply of water in the tissues would tend to hinder the reaction 'sugar→starch'; a more limited supply would tend to raise the concentration of the sugar so that it was maintained in the region of the critical concentration necessary for starch formation and would thus favour starch formation. Quite possibly plants subjected to short days do not pass quite as much water through the tissues as do control plants,[1] as the stoma are shut for a longer period. By raising the sugar concentration, starch formation would be favoured. In a recent paper Garner, Allard, and Bacon (11) have demonstrated practically that the sugar concentration of *Cosmos* plants was raised when the plants were subjected to shorter days, under which condition elongation is checked. Further, plants subjected to longer nights have longer time in which to carry out condensation. Harvey's (12) work, however, indicates that a dark period for translocation is not necessary. Practical tests, made by the writer, to see if there was any difference between the water-absorbing capacity of control and treated plants yielded negative results. Further experiments along similar lines are to be carried out with more succulent tissues, as those tests performed were carried out with parts of stems in which some lignification could not be avoided, other material not being available.

When the long-day elongation results (Garner and Allard) are considered it may be that weak artificial light (photosynthesis negligible), by opening the stoma and aiding transpiration, tends to hinder starch formation. Some tests made by the writer with a 40 c.p. light at 4 ft. gave results that, though not entirely satisfactory, were indicative that the stoma could be made to open by a comparatively weak light.

A series of hydrogen-ion-concentration determinations reported by Garner, Allard, and Bacon (11) indicate that elongation of the axis under artificially long days is correlated with high acidity. Mention may be made of the work of Compton (3), who demonstrated that the efficiency of the enzyme maltase depends upon the pH value of the substrate. It is, then, possible that the varying hydrogen-ion concentration found by Garner, Allard, and Bacon may help to control the activity of the enzymes affecting the sugar-starch ratio.

From a consideration of the growth results, it is now suggested that

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[1] It is assumed that none of the plants suffered from water-strain.

length of day affects the utilization of the food products by adjusting the sugar-starch reaction, and so controlling elongation. Garner and Allard state that 'Whilst photosynthesis is reduced by shortening the day, the power to elongate is further reduced', so that under short-day conditions the carbohydrate produced tends to give rise to a 'tuberized' structure, in many cases.

Such a theory finds some general support from other work upon growth, as that of Coville (4), who, by exposing plants to low temperatures, influenced elongation. His results are explained upon the basis of sugar production, caused by the low temperatures making contact possible between enzyme and carbohydrate to be hydrolysed.

Howard (15), in an extensive study of the rest period of plants, found that freezing and ether treatment favoured early growth. These results may also be interpreted on similar lines: the treatments tending to produce sugars necessary for elongation by making contact between enzymes and stored carbohydrate.

Klebs (20) has pointed out how the internal condition of the cells determines the form of a plant, or plant organ, and how the external factors may govern the internal conditions. It is therefore perhaps to be supposed that the effect of length of day upon growth is but another example of such phenomena.

VI. *Flower Production.*

Klebs (22), in his work upon *Sempervivum*, analysed the process of flowering into stages. Before flowering could take place he marked off a stage reached by the plant called 'ripe to flower'. One of the most interesting results obtained by shortening the daylight period is that this stage can be reached when the plant is exceedingly small. Control Soy Beans approximately 1 ft. high may be devoid of flower-buds, whilst tiny plants $3\frac{1}{2}$ in. high may bear pods under a six-hour day. Ripening of the fruits, the last phase of flower production, can also be accelerated.

Flowering may be prevented in 'short-day plants', e.g. *Cosmos*, by subjecting the plants to artificial long days. Garner and Allard maintained such plants in the vegetative state until they reached great heights. They have further demonstrated the fact that one part of a plant may be brought to the flowering stage whilst another branch of the same plant, subjected to different light conditions, continues vegetative growth.

Whilst evidence has been presented to show that shortening the days may accelerate flowering in one group of plants, the same treatment may retard flowering at different stages in the process of flower production, in another group of plants. This is clearly shown by considering some of the results obtained with the Gramineae.

Cocksfoot plants under nine and six hours' daylight did not reach the

'ripe to flower' stage, they did not 'shoot' out longer tillers. Timothy plants produced primordia—young glumes enclosing a group of meristematic cells, but development ceased at this stage.   Varieties of Oats were checked in a later stage, panicle exsertion being either hindered or prevented. Whereas it has here been so far only possible to carry out tests by shortening the day, abundant evidence exists to show that a group of plants (' long-day plants') can be made to flower early by lengthening the day.   The work of Adams (2) affords additional evidence to that of Garner and Allard in this respect.   Tjebbes and Uphof(33) have hastened flowering in bulbous plants, such as Tulips, Hyacinths, Crocus, by lengthening the days.

In discussing the results obtained with the grasses in an earlier part of this paper, it was noted that the various strains and pure lines of a species differed markedly in the extent of the range of daylight in which they could flower, late strains of our indigenous species being unable to flower when the light was but slightly reduced.   Possibly under longer artificial days these plants would again behave differently from the early strains.

When a large number of species are considered it is seen that there is a gradation from those only enabled to flower in a short-day range to those enabled to flower in a range of long days.   There does not exist, then, any hard and fast line separating short- and long-day plants.   In fact there are species which flower in such a wide range that it would be difficult to designate them either short- or long-day plants ; others, like *Tephrosia candida*, have a very narrow range (MᶜClelland (24)).

As has been previously pointed out by Garner and Allard, there would seem, from the point of view of length of day alone, to be no reason why many of the spring-flowering types should not flower in autumn and vice versa.   In annuals some vegetative growth is necessary before flowering can take place, and often sowing is late, so that flowering then tends to be delayed till autumn.   In woody perennials, e. g. fruit trees, autumn-formed buds do actually break into blossoms at once under particularly favourable temperature conditions.   It is not suggested that length of day is the only factor of phenological importance : due importance must be paid to other environmental conditions in considering flower production.   Eaton (5) has demonstrated the effect of temperature upon time of flowering in Soy Beans, for example.

VII. *Vegetative and Reproductive Activities.   Longevity.*

In annuals, biennials, and short-lived perennials the production of flowers is very closely related to the question of longevity ; vegetative growth ceases after the blossoming and ripening of the fruit and death follows. Biloxi Soy Beans subjected to the full daylight of a Cardiganshire summer remained green and healthy in October.   Plants subjected to a 12-hours

period had flowered and ceased vegetative activity and died. The controlling influence of length of day is further illustrated by referring to the results obtained with *Raphanus sativus* by Garner and Allard and the present writer. Plants of this species were made to resume vegetative growth by cutting down the daylight, new aerial leaf rosettes being formed upon flowering stalks. *Digitalis purpurea* resumed vegetative growth after flowering to a limited extent under short days.

Further evidence has been obtained to show that the length of day affects the balance between vegetative and reproductive growth.

A plant of the ' Tussock ' type of Cocksfoot (Bc. 914 (1) 6), after producing panicles on short stalks which gave rise to stamen-producing flowers, liberating much pollen, resumed vegetative growth. The glumes became elongated, flattened, and leaf-like structures. The palae also were not normal, becoming in some cases broader and less ' keeled ', and generally more leaf-like. The appearance of such panicles resembled that of a viviparous *Fescue ovina* panicle described by Jenkin (18). Ripening and normal seed production was greatly retarded and partially prevented, vegetative growth replacing the later stages of sexual reproduction.

The American workers have made Soy Beans, typical annuals, to flower early and produce seed by shortening the day ; on allowing these plants longer daylight, vegetative growth was resumed. The approach of natural short days produced further flowers on these newer branches. Asters have also been made to complete two flowering cycles in a period of four months by manipulation of the length of day.

The long-day plants which have missed a season's flowering in the tests carried out at Aberystwyth are apparently quite healthy ; it remains to be seen how, for example, the Montgomery clovers will behave from the longevity point of view. Record Oats under the short days remain as yet in the late tillering stage and are functioning as biennials or short-lived perennials.

VIII. *The ' Carbohydrate-Nitrogen Ratio' Theory of Flower Production.*

It is a general phenomenon that excess nitrogenous manure tends to favour vegetative growth and delay flowering. In 1897 investigation with Grapes by Müller-Thurgau (25) led to the view that nutrition was one of the most important factors governing fruit formation.

Klebs (22), working with *Sempervivum Funckii*, arrived at the conclusion that a piling up of carbohydrate food products favoured flower production ; he also concluded that a decrease in the water-supply and nitrogenous salts tended to assist flowering. High temperatures, causing increased respiration and the oxidation of carbohydrates, delayed flower production. No direct simple relationship between the light (measured by the product of intensity and time) and the production of flower primordia was

established for all conditions of the plant. The evidence, however, clearly pointed to the view that an increased concentration of carbohydrate favoured flower production. An increased concentration of carbohydrate in the leaves of Easter lilies, grown under artificial light, has been found to be correlated with early flowering (Hendricks and Harvey (14)).

Harvey and Murneck (12), by chemical analysis, have studied the relation of carbohydrates and nitrogen present to the behaviour of Apple-spurs, and they conclude that ' whilst no conclusion is yet drawn as to the existence of a definite causal relation between the carbohydrate-nitrogen ratio and the performance of a plant organ, it is believed that the relation as indicated by this expression should be given an important place amongst the group of controlling factors ' to be considered. Their results tend to show that fruit-bud formation takes place when the carbohydrate-nitrogen ratio lies within definite limits ; the individuality of the fruit-spur is also stressed.

Kraus and Kraybill (23), working with Tomatoes, have found that flowering and fruiting can only take place when the carbohydrate-nitrogen ratio lies between certain limits. Analysis of stems showed that there is more carbohydrate near the apex and more nitrogen farther down ; a correlation between the change of the carbohydrate-nitrogen ratio and the sequence of reproductive activities would seem to exist, whether this ratio gradient is the cause or the effect of the reproductive sequence.

Petri (26) has investigated the nitrogen nutrition of Olives, and found that fertile branches contain a higher nitrogen percentage than do non-fruitful branches. The results of recent work on pruning of fruit trees by Pickering and the Duke of Bedford (27) can be viewed from the standpoint of the carbohydrate-nitrogen ratio, pruning young trees removing too much potential carbohydrate, and so limiting fruit production.

From a consideration of the above evidence an hypothesis can be constructed, namely, that for flower formation to occur the plant, or part of the plant, must reach a stage of metabolism at which the ratio of carbohydrate to nitrogen present lies between certain definite limits. Plants whose chemical content fulfils such conditions would be ' ripe to flower ' ; an excess of either food substance, as well as a deficiency, by upsetting the ratio, would retard flower formation.

It is necessary to consider the plausibility of this hypothesis from the results obtained by altering the length of day. When short-day plants are considered, some practical evidence exists in favour of this theory. It has been shown by Garner, Allard, and Bacon (11) that subjecting these plants to short days not only checks elongation and produces flowers, but raises the carbohydrate content. Under long days such plants continue elongation and vegetative growth without flowering, presumably because the carbohydrate-nitrogen ratio is too low for flower production.

Extremely short days cause the starch content of some long-day plants to be exceedingly high, as seen in tuberized structures ; it is suggested that in such cases there is too much unsuitable carbohydrate to permit flowering. Under longer days some long-day plants (e. g. Radish) have been shown to possess more soluble carbohydrate (sugar) and to produce elongated flower-axes and later flowers. It is suggested that in those inter-mediate types where alteration of the length of day does not alter the time of flowering the ratio of carbohydrate to nitrogen is not seriously affected. It must be remembered that length of day can probably affect the nitrogen factor by controlling transpiration, as well as the nature and quantity of carbohydrate.

In summarizing this section it may be that the complex length of day factor operates by adjusting the quantity and quality of the carbohydrate, and, by its transpiration effect, the nitrogen content. Such a mechanism would seem possible and would not be in contradiction to existing theories of flowering.

Enough direct experimental evidence upon the effect of length of day upon internal chemical composition does not yet exist. It would seem, however, that an explanation of the results is possible without supposing that the actual duration of the periods of light and darkness are the operating factors. Plants grown in continual artificial light by Harvey (13), with no period of darkness at all, have completed a normal life-cycle. It would therefore appear that a period of darkness is not really necessary ; because of this evidence less importance must be attached to the view that it is the ' length ' of the periods of light and darkness alone that matter.

Whatever interpretation may ultimately be found to be most satisfac-tory the results remain clear—a simple adjustment of the natural daylight period produces remarkable changes in the behaviour of plants ; the prac-tical importance of the results from an economic standpoint is independent of the interpretation.

Economic Considerations.

From the point of view of the practical plant breeder difficulty is often experienced in bringing about pollination and fertilization between a normally early and a late variety. By controlling the length of day Emerson (6) has been able to produce flowering in *Teosinte (Reana luxurians)* at a suitable time for carrying out cross-pollination with Maize.

The reaction of different strains or pure lines to the different lengths of day affords an indication of the potential behaviour of the plants in other latitudes. For example, Wanser (34), working with Wheat, considers photo-periodism a determining factor in acclimatization.

As the nature of the crop desired, vegetative bulk, or quick seed pro-

duction varies, so ought also the time of planting to vary. The longer the period of growth the greater the vegetative yield produced, but for quick seed production sowing may be delayed until just before that part of the season which will, by means of its length of day, produce flowers.

Conclusion.

The effect of length of day upon flower production and upon growth throws further light upon plant periodicity. 'Periodicity', like other phenomena when subjected to analysis, yields results which show that by manipulation of the external factors sexual reproduction and growth-form can be controlled by environmental conditions.

· SUMMARY.

1. The behaviour of a number of plants under short periods of natural illumination has been studied. Particular attention has been paid to herbage plants.

2. By exposing plants of Soya Bean (three varieties), Chrysanthemums, and Runner Beans to these shortened days, flowering has been accelerated. Growth also has been modified and controlled.

3. By exposing other plants, Red Clover (three varieties), Gramineae (several species, approximately twenty types), Radish, to these conditions, flowering has been retarded. Under shorter days the season habit-change has not taken place.

4. Foxglove gave an intermediate type of result—although growth of the flowering axis is reduced by very short days and by very long days the time of blossoming is not materially altered.

5. The effect of length of day upon growth has been observed. Elongation and tuberization as expressions of the manner in which food products are utilized have been studied. Some discussion of underlying chemical changes has been attempted.

6. The actual results are in very close agreement with those of Garner and Allard.

7. Some discussion of the length of day factor is given. Consideration of this complex factor shows that it consists of a number of units, all of which affect the plant's activities. It may be that length of day brings about fine adjustments in the amount of water passing through the tissues, and operates by affecting the sugar-starch reaction. The results are not completely explained by considering photo-reduction only.

8. The carbohydrate-nitrogen ratio theory of flowering has been briefly outlined and considered from the point of view of these and other similar results.

9. Economic possibilities arising out of a consideration of the results are briefly touched upon.

10. The results emphasize the plasticity of the plant. Evidence has been presented to show the controlling influence of environment on internal chemical composition, plant form, behaviour, and periodicity.

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DESCRIPTION OF PLATES XVII–XIX.

Illustrating Mr. Tincker's paper on the Effect of Length of Day upon the Growth and Reproduction of some Economic Plants.

PLATE XVII.

Phaseolus multiflorus, Ref. No. As. 74.

Fig. 1. Young *multiflorus* plants photographed when treatment began. 1/6/24.

Fig. 2. Left to right, 12-hours, 9-hours, and 6-hours plants, with control on the right. Photographed 30th June, 1924.

Fig. 3. A 9-hours short plant with tall control plant. The 9-hours plant flowered four days earlier than control. Flowers can be seen on the short plant, buds on the tall control.

Fig. 4. Showing two thick ' tuberized' roots of the 12-hours plants on the left and the fibrous roots of the control on the right. Photographed 29th August 1924. Much starch was present in the cells of the thickened roots.

Fig. 5. Swollen nodes of a 12-hours and a 6-hours plant showing signs of ' tuberization'. Much starch was present in the thick petioles.

Chrysanthemum (var. *Mrs. William Buckingham*).

Fig. 6. Photographed 15/8/24. Treatment commenced 9/5/24. Left to right, one replication of 12-hours, 9-hours, and 6-hours plants, and control (full day). No buds on control 15/8/24; controls flowered normally in November—2½ months later than 12-hours plants.

Anthoxanthum odoratum—Sweet Vernal Grass.

Fig. 7. Narrow-leaf type Sweet Vernal Grass, Ref. No. 15 Bs. (1) 1. Photographed late August; treatment started 1st February. Left to right, 12-hours, 9-hours, and 6-hours plants, and tall control. Though the seed was shed from the control, only one poor panicle had appeared on the 6-hours plants, which were very leafy.

Alopecurus pratensis—Meadow Foxtail.

Fig. 8. ' Early type' Meadow Foxtail, Ref. No. 277 Bh. 7. Photographed 19/5/24. Left to right, 12-hours, 9-hours, and 6-hours plants, and control. The 6-hours plants produced a few poor panicles. Treatment commenced 1/2/24.

Fig. 9. ' Late Pasture type' Meadow Foxtail, Ref. No. 278 Bh. 21. Photographed 3/6 24. Left to right, 12-hours, 9 hours, and 6-hours plants and control. No panicles on the 6-hours plants. The 9-hours plants also produced less panicles than did the early Hay type under nine hours' daylight.

Dactylis glomerata—Cocksfoot.

Fig. 10. ' French Hay' type Cocksfoot, Ref. No. 812 Bc. (1) 24. Left to right, 12-hours, 9-hours, and 6-hours plants, and control. Photographed 29 7 24. Ripe seed on control; no panicles all the season on 9-hours and 6-hours plants.

PLATE XVIII.

Fig. 11. ' Late indigenous' Cocksfoot, Ref. No. 828 Bc. (1) 13. Left to right, 12-hours, 9-hours, and 6-hours plants, with tall control which produced ripe seed. Photographed 5/8/24.

Fig. 12. Cocksfoot, ' Tussocks' type, Ref. No. 914 Bc. 1) 6. At the commencement of treatment all equal.

Fig. 13. Cocksfoot ' Tussocks ' type, Ref. No. 914 Bc. (1) 6. Left to right, 12-hours, 9-hours, and 6-hours plants, with tall control plant. Photographed 15/8/24.

Phleum pratense—Timothy.

Fig. 14. ' Early type' Timothy, Ref. No. Bd. 92. Showing left to right, 12-hours, 9-hours, and full day control plants in panicle. Photographed 25/6/24.

Fig. 15. ' Late type Timothy', Ref. No. Bd. 48. Left to right, 12-hours, 9-hours, and 6-hours plants, and tall control in panicle (seed ripening). Note that the 6-hours plant ' shot up ' as if about to flower, but flowering was prevented in this late stage. Photographed 29/8/24.

Avena sativa—Oats.

Fig. 16. Ceirch-du-bach Oats, Ref. No. 952/4 Cc. Photographed 3/6/24. Left to right, 12-hours, 9-hours, and 6-hours plants sown 1/2/24, treatment commenced at sowing.

Fig. 17. Identical plants as in Fig. 16. Photographed 15/8/24. Left to right, 12-hours, 9-hours, and 6-hours plants, and control with ripe grain. Note the particularly ' leafy' appearance of the 6-hours plants where tillering was prolonged slightly.

Trifolium pratense—Red Clover.

Fig. 18. English Broad Red Clover, Ref. No. Aa. 505, showing control in flower and 6-hours plants on 3rd June in winter habit.

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Fig. 19. American Medium Red Clover, Ref. No. Aa. 510. Left to right, control full day plant with some ripe flower-heads ; 12-hours plant, some small flower-heads ; 9-hours plant, semi-erect habit and a few poor flower-heads ; 6-hours plant, prostrate in winter habit. Note gradation of response varies as length of day. Photographed 28th July 1924.

Fig. 20. Montgomery Late Flowering Red Clover, Ref. No. Aa. 616. All these plants are propagants of one parent. Left to right, 12-hours, 9-hours, 6-hours, and control plants starting to become erect. Photographed 18th July 1924.

Fig. 21. Same plants as Fig. 20. Photographed 2nd August 1924, showing 12-hours, 9-hours, and control plants flowering. The 6-hours plants (not in this photograph) were also in the winter habit quite prostrate.

PLATE XIX.

Raphanus sativus—Scarlet Globe Radish.

Fig. 22. A box of 6-hours plants in rosette habit and a box of control plants flowering (10 in.) 3rd June 1924. Six-hours plants did flower, 3rd July 1924, at half the height of these controls.

Digitalis purpurea—Foxglove.

Fig. 23. Left to right, 12-hours, 9-hours, and 6-hours and control plants. Its. approx. 11 in., 6 in., 4 in., and 8 in. Control tall. Photographed 19/5/24. Treatment started 1st Feb. on equal ' rosettes '.

Fig. 24. Left to right, 12-hours, 9-hours, and 6-hours plants. The plants reached a height of 50 in., 12-hours plants ; 34 in., 9-hours plants ; 20 in., 6-hours plants ; and control (not in photograph), 30 in. There was very little difference in time of flowering, however ; compare 9-hours and 6-hours plants in photograph. 3/6/24.

Helianthus tuberosus—Artichoke, and *Brassica oleracea*—Cabbage.

Fig. 25. Artichokes, Ref. No. Ds. 24. Showing left to right, 12-hours, 9-hours, and 6-hours plants, also control. Note differences in height. Photographed 21st May 1924.

Fig. 26. Same plants as Fig. 25. Photographed 18th June 1924. Left to right, 12-hours, 9-hours, and 6-hours plants, also tall control.

Fig. 27. The produce of these plants ; left to right, 12-hours, 9-hours, and 6-hours plants, and small poor tubers of control. September 1924.

Fig. 28. Cabbage, Ref. No. Ds. 26. Left to right, 12-hours, 9-hours, and 6-hours plant, also control plants. Treatment started on equal plants 8th February 1924. Photographed 29th August 1924.

TINCKER— GROWTH & LENGTH OF DAY.
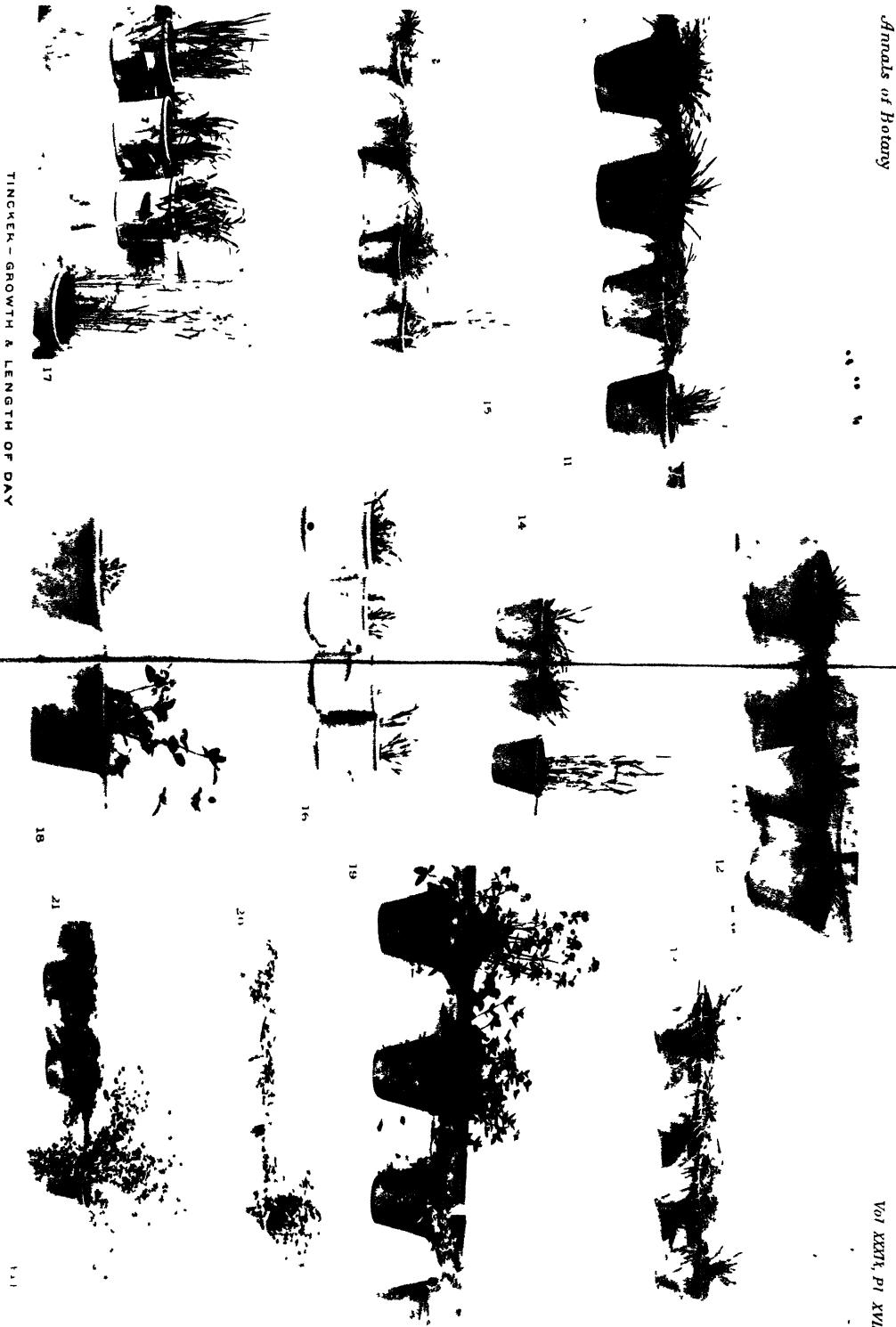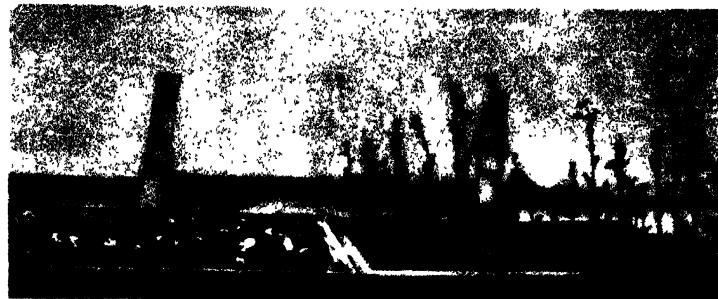
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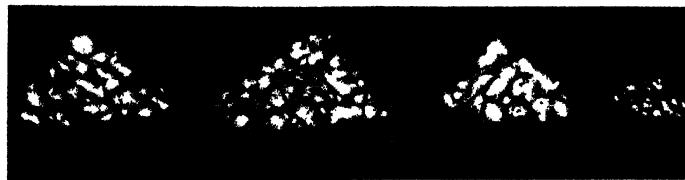
TINCKER—GROWTH & LENGTH OF DAY.

The Plant Cuticle.

II. A Macrochemical Study.

BY

BEATRICE LEE.

INTRODUCTION.

LITTLE continuous work has been carried out upon the chemistry of cuticle, most observations being incidental to studies of this layer carried out from the botanical side. Chemists are now studying the cotton hair with technical methods and machinery at their disposal (1, 4, 5) and their results should be of great value to the botanist. Botanical work is limited by the difficulty of obtaining large supplies of material in a form suitable for analysis. The work that is described in this paper, small though the amounts of cuticle under treatment may seem in comparison with those used in the work upon cotton wax, could never have been carried through in the last few years had not grants in aid enabled assistants to be enlisted in the tedious process of removing and preparing the cuticular layer. Fargher and Higginbotham (4) have recently published details of analytical work upon the constituents of waxes, in particular cotton wax, where from c·1 to c·2 grm. of substance have been employed. The development of technique along these lines will be of material help to the botanical investigator. In the preliminary study little use could be made of such technique. as it was felt to be more essential to determine quantitatively the amount of certain categories of chemical substances present as fats, oxy- and normal fatty acids, &c., and to obtain data as to certain chemical and physical properties, such as degree of unsaturation and solubility, rather than to commence the

difficult task of identifying specific chemical constituents. The result is that, by studying both the normal mature cuticle and the less-developed layer found upon young shoots grown under forcing conditions, certain data can be obtained which seem to throw light upon the essential chemical nature of cuticle and the chemical changes which are involved in its formation.

I. HISTORICAL.

During the nineteenth century several attempts were made to analyse the cork and cuticle of plants. These two layers can resist the action of soil water and other agents causing decay of plant tissues, as is seen in certain coals that have been found to consist almost entirely of layers of cuticle. The respective powers of resistance of cork and cuticle to different reagents were tested in these early investigations. Both were found to be affected by heating in strong alkaline reagents, and it was concluded that they contained similar substances: suberin and cutin.

Eugene Gilson (7, see also 10), using the method given in Table II (p. 762), analysed the corky tissue of *Quercus Suber*, L. He saponified the finely ground, dried cork and then separated, from the solution of fatty soaps, three fatty acids: phellonic, suberinic, and phloionic. Phellonic acid is insoluble in cold alcoholic caustic potash and so separates out as a precipitate which, on purification, gives a crystalline fatty acid with characteristic colour reactions, producing a violet colour when moistened with a dilute alcoholic solution of iodine followed by concentrated sulphuric acid. The solubility of this acid changes rapidly on exposure to heat or light.

Suberinic acid is soluble in alcohol, ether, and chloroform, but insoluble in petrol ether, in this resembling the oxy-fatty acids of Fahrion (3), which are also insoluble in petrol ether but soluble in ether.

Phloionic acid is peculiar in being soluble in alcohol, but only very slightly so in ether and chloroform, and also a little soluble in hot water.

Gilson established the presence of glycerol in his solution after acidifying the alcoholic solution of potassium soaps and filtering off the fatty acids precipitated.

The results obtained by Fremy and Urbain (6) in their examination of cuticle are partly comparable with Gilson's. On saponification they find that 'cutose', the outermost layer of the cuticle, free from cellulose, gives two fatty acids: stearocutic, a white solid, melting at 76° C.; and oleocutic, a liquid fatty acid. By heating up to 100° C. and exposing to the light, they tried to imitate the conditions under which the fatty acids would exist in the cuticle, and so altered their solubilities and melting-points, the acids becoming insoluble in alcohol, ether, and cold alkaline solutions, and the melting-point of stearocutic acid rising to 96° C.

The recent work of Fargher, Probert, Clifford, and Higginbotham in

Manchester, on the constituents of the fats and waxes of American and other cottons, is comparable with that on other cuticular extracts, since the fatty substances extracted by their different solvents will come mainly from the cuticle of the cotton hair. Clifford and Probert (1) describe the constituents of the wax of American cotton as being wax alcohols, montanyl $C_{28}H_{58}O$, and gossypyl $C_{30}H_{62}O$; glycols and sterols, chiefly sitosterol, with possibly another new sterol $C_{34}H_{58}O$; acids, among which are palmitic, stearic, carnaubic, gossypic, montanic, and the unsaturated acid, oleic. The first three acids occur as glycerides and are probably also combined with phytosterols. The wax also contains liquid hydrocarbons and a complex mixture of resin esters and alcohols. Glycerol was definitely present.

II. Experimental Methods.

It is difficult to obtain cuticle free from cellulose from the underlying cells, xylem vessels, cell contents, anthocyanins and other colouring matters. Unsuccessful attempts were made to collect material by peeling off the cuticle of different leaves, and, finally, it was obtained by using the flower petals of chrysanthemums, garden roses, and *Scilla nutans*, L., and by stripping off the cuticle from the petioles of rhubarb. The petals were gathered while fresh and dried at room temperature. Usually they were put into a steam oven for two or three hours at a temperature of 50° C. before grinding to a powder.

In the earlier analyses the powdered cuticle was extracted with dilute hydrochloric acid to remove the anthocyanins, but this left the cellulose and protoplasmic impurities. A solution of zinc chloride in hydrochloric acid was next used, this being a cellulose solvent. This treatment removed most of the cellulose and the colouring matters, but it also affected subsequent extractions. The unstable compounds in the cutin reacted either with the zinc chloride or the hydrochloric acid, so that the percentages of soaps and free fatty acids obtained from the treated cuticle were not those of the original material.

In the method finally used the petals or rhubarb skins were dried and powdered, and after powdering put back into the steam oven for about three hours. The dry weight of the cuticle used was found by calculating the loss in weight of a small quantity, when heated to constant weight in a steam oven, and from this calculating the loss in weight of the whole.

A weighed amount was then extracted thoroughly with chloroform in a large Soxhlet funnel made in the workshops of Rowntree's Cocoa Works for cocoa-bean fat extraction, and kindly lent to us by their chemical department. Chloroform was used because it does not extract anthocyanin and because it is not inflammable. Although this first fat extract is free from anthocyanin, it contains resinous impurities, such as Clifford, Higgin-

botham, and Fargher conclude to be extracted by the chloroform, together with the fat and wax of their cotton. The chloroform was removed from the fat by distillation and drying in a vacuum desiccator over potassium sulphate.

The residual cuticle was dried and weighed and extracted with ten per cent. hydrochloric acid in a large glazed earthenware pan until, on washing with water, no more colour came away. The temperature of these extractions was kept below 60° C. The cuticle was washed with water, until free from acid, dried and weighed. The dry weight thus obtained, together with that of the fat extracted, was used as the basis for the percentage weights of the different extracts, since this represents the cuticular material as free as possible from impurities.

The residue, now hard and tough, was ground through a hand-mill and the powder saponified with a three per cent. solution of caustic soda. or potash, in alcohol. After saponifying for three hours, the alcoholic solution was filtered hot and the residue exhaustively extracted with alcohol. These extracts were added to the first, and the whole reduced to a small volume by distilling off the alcohol. This gave the second extract of cuticle, containing fats and fatty compounds insoluble in chloroform.

The residue no longer gave a fat reaction with Sudan III, but on extraction with water yielded large quantities of tannin-like products. These were dark brown and brittle ; they contained a small amount of nitrogen.

After the aqueous extraction, the residual material gave a strong cellulose reaction, although it contained a proportion of a substance that is unaffected by iodine with sulphuric acid or by chloriodide of zinc. After from twelve to twenty-four hours' hydrolysis with ten per cent. sulphuric acid, about thirty per cent. of the residue remained unhydrolysed. This gave no fat nor cellulose reactions, and corresponds with that layer which is so difficult to remove in microscopic sections (see former paper (9)). It contained nitrogen, so that there is some other compound present in addition to any resistant anhydrides of cellulose.

When sections of petals are left in strong sulphuric acid and subsequently washed with water, only the cuticle remains, so that this residue which resists hydrolysis must be in the cuticle as a basis to the fatty deposit.

1. *Examination of Chloroform Extract.*

In preliminary experiments, the chloroform soluble fat, after saponification, was found to contain saturated and unsaturated acids, which were separated by making use of the different ·solubilities of their lead salts in ether. Small quantities of volatile fatty acids were distilled off in steam.

The purpose of this examination is to discover the degree of oxidation and condensation undergone by the fatty compounds in the chloroform soluble fat in the cuticle, and, as a consequence, the change in its solubility.

Assuming that the origin of many of these fatty substances is in meristematic regions of the plant, it is possible to compare them, as they were when they first accumulated at the surface of the protoplasmic membranes in the vacuolating cells, with them as they are in the cuticle. Fat extracted from growing shoot tips of *Vicia Faba* by R. M. Woodman, in this laboratory, had an iodine value of about 73, that from the root-apex of 114, indicating the presence of a large proportion of unsaturated compounds. In the tables following, it will be seen that the highest iodine value obtained, 90, is for the fat from the forced rhubarb cuticle. That of outdoor-grown is 54. The fats exposed to the oxidizing and drying conditions of the open air become saturated quicker than those exposed to the damp atmosphere of the forcing sheds.

This change in the ratio of unsaturated to saturated compounds means that, as the amount of unsaturated fat decreases, that of ' oxy-fatty acids' will increase.

Linseed oil is known to set to 'linoxyn' by oxidation. The real oxygen absorption is greater than the apparent, because, at the same time as increase in weight is taking place, volatile decomposition products are evolved. Mulder found that, of these products, the greater part consisted of water, carbon dioxide, and acetic acid. Formic, acrylic, and butyric acids are there in smaller quantities.

In one of his papers on ' The Mechanism of Oxidation of Drying Oils', Coffey (2) suggests that a primary oxidation of linseed oil is probably a case of molecular autoxidation, in which a molecule of O_2 is added at the double linkages.

$$\begin{matrix} -CH \\ \parallel \\ -CH \end{matrix} + O_2 \quad \rightarrow \quad \begin{matrix} -CH-O \\ | \quad | \\ -CH-O \end{matrix}$$
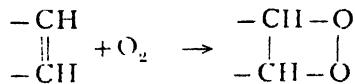
This process is followed by the addition of more oxygen and the splitting off of volatile acids as a consequence. He noticed that volatile acids only came over in later stages of the oxidation process.

The oxidation processes of the fats in the cuticle are probably analogous with those described by Coffey in this and other papers (2). The volatile products, carbon dioxide, acetic acid, and water, which are formed in the first stages, are lost to the air, the result of their loss being seen in the formation of the comparatively rigid cuticle. In an experiment described later (see p. 760), in which cuticular fat is heated for several weeks at a temperature of about 60° C. in a flask with only a small outlet tube, the fat set to a brittle varnish ; and when the stopper was removed, volatile

substances were detected by their smell. Evidently in this fat complete oxidation only takes place at higher temperatures than those normally experienced by the plant.

A sample of the chloroform soluble fat was saponified by boiling with three per cent. alcoholic NaOH. The resulting solution was evaporated nearly to dryness and the product taken up in water, after which an extraction with ether removed unsaponifiable material. Then the quantity of 'oxy-fatty acids' present was found using Fahrion's method (3), in which he separates these from the 'normal fatty acids'. The aqueous solution of sodium soaps was acidified with dilute phosphoric or hydrochloric acid. The cold suspension of acids was extracted with petrol ether (B.P. 40°–60°C.), which dissolved the normal acids and left in suspension the oxy-acids. After evaporating to dryness, the oxy-acids were extracted with warm alcohol. The alcoholic solution, on evaporation to dryness, yielded oxy-acids. These were dried and weighed. In some cases the suspension of oxy-acids was removed by filtration, and, after thorough washing with cold water to remove excess acid, was dried at a low temperature in a vacuum desiccator over anhydrous calcium chloride. This method was used because cuticular fatty acids have low melting-points, and, after melting, become sticky and difficult to handle. Whenever possible, drying processes were carried out at room temperature or below 50° C.

An examination of the acid value and the saponification value of this fat, together with its percentage of ash, gives some indication of the amount of fat present as free fatty acids or as soaps of fatty acids. The quantity of ash obtained has been too small to permit of a quantitative analysis of the bases present.

The solubilities of the fatty compounds should change when they are exposed to the oxidizing and drying effects of the sun and air. The solubility of the fat in chloroform was determined immediately after extraction, and then, in order to find out whether light and heat effect any change, the following experiment was made:

Thin layers of the fat were placed on the bottom of Erlenmeyer flasks by dissolving a small quantity in chloroform and then evaporating off the solvent. Four such flasks were prepared. Two were put into a bucket of water at a constant temperature of 23° C. in a room lit continuously by electric light, one flask being covered with tinfoil to exclude all light, and the other left so that it received continuous light. The other two stood in the daylight, one on the window-sill at an average temperature of 17° C., and the other on an electric heater at an average temperature of 60° C. After four weeks, the solubilities of the fats in these four flasks were again determined and the results tabulated as grm. of fat soluble in 100 grm. of solution.

2. *Saponified Extract of Potassium Soaps.*

The alcoholic extract of potassium soaps was evaporated down to a small volume, taken up in warm water and then extracted with ether in order to remove the unsaponifiable material. After acidifying the remaining solution with 50 per cent. phosphoric acid, a separation of the normal and oxy-fatty acids, similar to the previous one, was made.

The aqueous solution, after the removal of the fatty acids, was tested for glycerol, the acrolein test with potassium hydrogen sulphate and the benzoyl chloride test, which gives a crystalline precipitate of tribenzoate of glycerol, being used.

A summary of the complete examination of cuticle is given in the following table:

TABLE I. *Raw Material.*

1. Dried and weighed.
2. *Hot chloroform extraction*—Extract A.
3. Residue freed from CHCl₃ and dried.
4. Extraction with 10 per cent. HCl at 60° C. until all colour removed. Washed residue with warm water to remove HCl. Dried and weighed.
5. *Saponification* with 3 per cent. alcoholic NaOH—Saponified extract B.
6. Residue extracted with warm water. Aqueous extract dried and weighed. Tannin and nitrogen tests made.

A. *Examination of Chloroform Extract.*

1. Iodine value.
2. Acid value.
3. Saponification value.
4. Ash.
5. Saponified with 3 per cent. alcoholic NaOH. Distilled off alcohol and dissolved residue in water. Ether extraction to separate *unsaponifiable material.*
6. Fatty acids freed with 50 per cent. HCl.
   (*a*) Petrol ether extraction to separate *normal fatty acids.*
   (*b*) Residue filtered; precipitate washed with warm distilled water and dried. This yields *oxy-fatty acids.*

B. *Treatment of Saponified Extract.*

1. Alcohol removed by distillation. Residue dissolved in water and extracted with ether to separate *unsaponifiable material.*
2. Fatty acids freed with 50 per cent. HCl.
   (*a*) Petrol ether extraction to separate *normal fatty acids.*
   (*b*) Residue filtered; precipitate washed with warm distilled water and dried. This yields *oxy-fatty acids.*

N.B.—NaOH used was specially pure and prepared from metallic sodium. Absolute alcohol was used in the later experiments for the preparation of the 3 per cent. alcoholic NaOH.

III. EXPERIMENTAL RESULTS.

The first method chosen for a preliminary examination of cuticle was the one used by Gilson (7) in his analysis of the dried cork of *Quercus Suber.*

Details of his method can be obtained from his paper, but briefly it is as follows :

TABLE II. *Powdered Cork.*

Saponification with 3 per cent. alcoholic KOH. Filtered hot and filtrate left to stand
   24 hours. Filtered :
   (1) *Precipitate I* contains *phellonic acid.* M.P. = 95–6.
   (ii) *Solution II* acidified with HCl :

(i) *Precipitate A'.*	(ii) *Solution B'.*

Converted into K soaps. Alcoholic solution of soaps left for two days.	Contains glycerol as shown by :—
	   (*a*) Acrolein test.
	   (*b*) Benzoyl chloride test.

(i) *Solution C'.*	(ii) *Precipitate D'.*
Acidified with HCl. Contains *suberinic acid*—semi-liquid.	Dissolved in water. Acidified with HCl. Contains *phloionic acid.* M.P. 120–1.

An analysis of cork of *Quercus Suber*, using this method, yielded similar results to those described by Gilson, although the tests for glycerol were not as decisive as he states his to have been. This method was next used for an examination of the cuticle of different flower petals.

1. *Chrysanthemum petals.*

179 grm. of chrysanthemum petals, air-dried and powdered, were first extracted with hot 5 per cent. hydrochloric acid and warm water until no further colour showed in the water, and then washed until free from acid, and dried. The weight, after drying, was 59·5 grm. This residue was extracted with ether, and the ether extract, evaporated and dried, yielded 17·4 grm. of a dark-brown fatty substance. The residue of the petals was saponified with 3 per cent. alcoholic KOH. The precipitate I of Gilson separated out, but gave none of the phellonic acid reactions. The acids obtained after acidifying solution II had no resemblance to the corresponding cork acids, being almost insoluble in ether: the cork acids are soluble in ether, though phloionic acid only with difficulty. Solution B' gave negative reactions to the tests made for glycerol.

The ethereal extract of fats was saponified with 3 per cent. alcoholic potash. The acids obtained on acidification have no phellonic acid reactions and had little resemblance to suberinic or phloionic acids. Again tests for glycerol gave negative results.

2. *Petioles of Rhubarb leaves* (1).

The cuticle was stripped from the leaf-stalks of rhubarb grown out of doors and scraped free from any adhering vascular tissue. It was then dried and 150 grm. saponified with alcoholic potash.

Precipitate I gave no reactions like those of phellonic acid. The filtrate gave tannin reactions with bromine water, formaldehyde, and lead acetate.

An extraction of solution II with ether before acidification gave, after evaporation, a small quantity of unsaponifiable material. This gave some of the reactions of phytosterol, but was never obtained in a sufficiently pure condition to give a melting-point. From the acids freed from this solution a mixture, comparable with suberinic acid, was obtained, with an iodine value of 65·6. Precipitate D' was a white substance crystallizing from alcohol in needle-shaped crystals which were insoluble in ether, acetone, and chloroform, and had a melting-point below 100° C. This is too low for phloionic acid, which melts at 120–1° C.

The results obtained from the examination of the chrysanthemum petal cuticle and the rhubarb skin emphasize the following two facts: (1) Among the fatty acids present in cuticle there is no phellonic acid. (2) Glycerol is not present in a form that can be directly identified.

The method substituted for Gilson's is given in Table I on p. 761.

Four lots of cuticle have been examined using this method. Two of these four, the cuticle of rose petals and chrysanthemum petals, underwent this treatment without any modifications, and the results are given in Table III.

TABLE III.

	Rose Petals.	*Chrysanthemum Petals.*
Weight of dried petals	627 grm.	724 grm.
Weight after HCl—residue + fat	189·6 ,,	282 ,,
A. *Weight of fat*	23·1 ,, [12 %]	47·0 ,, [17 %]
(Percentage calculated on weight after HCl.)		
a. Iodine number	70·6 ,,	57·3 ,,
b. Acid value	35 ,,	55 ,,
c. Saponification value	275 ,,	330 ,,
d. Ash content	0·62 %	2·0 %
e. Solubility in $CHCl_3$	9·5 grm. in 100 grm. solution	—
(i) After heating at 60° C.	3·05 ,, ,, ,,	—
(ii) After light at 17° C	18·0 ,, ,, ,,	—
(iii) After continuous light at 23° C.	8·5 ,, ,, ,,	—
(iv) After continuous dark at 23° C.	10·0 ,, ,, ,,	—
f. (i) Unsaponifiable material	3·6 grm. [60 %]	4·5 grm. [12 %]
(ii) Normal fatty acids	in 6 ,, 0·83 ,, [14 %] in 36 grm.	16·3 ,, [45 %]
(iii) Oxy-fatty acids	1·52 ,, [25 %]	5·8 ,, [16 %]
B. *Saponification with 3 per cent. alcoholic KOH.*		
Weight of residue saponified	166 ,,	235 ,,
(i) Unsaponifiable material	0·9 ,, [6 %]	3·7 ,, [15 %]
(ii) Normal fatty acids	0·14 ,, [1 %]	1·2 ,, [5 %]
(iii) Oxy-fatty acids	14·7 ,, [93 %]	20·36 ,, [80 %]
C. *Residue A. (after saponification)*	133 ,,	—
Aqueous extract	63·7 ,,	97·5 ,,
N. number of acid precipitate	2·1 ,,	—
D. *Residue A*	69 ,,	96·6 ,,
N. number	2·7 ,,	4·1 ,,
Percentage hydrolysed	66·3 ,,	82·9 ,,
E. *Residue A 2* (unhydrolysed).		
N. number	3·2 ,,	4·6 ,,

The same method was used for a second analysis of cuticle from the petioles of forced and outdoor-grown rhubarb, but during this analysis two alterations were made in the use of solvents. Pure caustic soda, made from metallic sodium, was dissolved in a small quantity of water and made up to a 3 per cent. alcoholic solution with absolute alcohol and used instead of the alcoholic KOH as is noted in the table. The ether used for the extraction of the unsaponifiable material was purified by distilling from caustic potash.

TABLE IV.

	Forced Rhubarb.	*Outdoor Rhubarb.*
Weight of dried skin	517　grm.	495　grm.
Weight after HCl—residue + fat	271·4　,,	291　,.
A. *Weight of fat*	16·6　,,	10·77　,,
(Percentage calculated on weight after HCl)	5·9 %	3·6 %
a. Iodine number	90·5	54·3
b. Acid value	70	60
c. Saponification value	337	870
d. Ash content	2·35 %	0·85 %
e. Solubility in CHCl₃	5·85 grm. in 100 grm. solution	4·4 grm. in 100 grm. solution
(i) After heating at 60° C.	1·5 grm.	—
(ii) After light at 17° C.	8·9 ,,	—
(iii) After continuous light at 23° C.	10·2 ,,	—
(iv) After continuous dark at 23° C.	11·3 ,,	—

	Saponified with alcoholic NaOH.	Saponified with alcoholic KOH.
. (i) Unsaponifiable material	0·63 grm. [12·6 %]	3·14 grm [52·3 %]
(ii) Normal fatty acids	in 5 grm. 2·27 , [45·6 %]	in 6 grm. 1·78 ,, [29·6 %]
(iii) Oxy-fatty acids	0·65 ,, [13·0 %]	1·24 ,, [20·7 %]

B. *Saponification with 3 per cent. alcoholic KOH.*

Weight of residue saponified	255　grm.	—
(i) Unsaponifiable material	8·7　,, [43·9 %]	—
(ii) Normal fatty acids	3·0　,, [15·2 %]	—
(iii) Oxy-fatty acids	8·1　,, [40·9 %]	—
C. *Residue A* (after saponification)	229　,,	.
Aqueous extract	59　,,	—
D. *Residue A* 1	165　,,	

TABLE V.

	Forced Rhubarb.	*Outdoor Rhubarb.*
Weight of dried skin (air-dried)	111　grm.	114·4　grm.
Weight of dried skin (dry weight)	94　,,	100·5　,,
Weight after HCl (dry weight)	40·3　,,	51·28　,,
Weight of residue + fat	42·86　,,	53·52　,,
Weight of fat	2·56　,,	2·24　,,
Percentage weight calculated on weight after HCl	5·97 %	4·19 %

Saponification with 3 per cent. alcoholic NaOH.

	Forced		*Outdoor*	
Weight of residue saponified	40·3　grm.		51·28　grm.	
(i) Unsaponifiable material	0·076 ,,	[2·1 %]	0·107 ,,	[5·0 %]
(ii) Normal fatty acids	1·09 ,,	[30·4 %]	0·038 ,,	[15·3 %]
(iii) Oxy-fatty acids	2·42 ,,	[67·5 %]	1·715 ,,	[79·8 %]

Two more quantities of forced and outdoor-grown rhubarb cuticle were analysed, using the same method with these two alterations. The results of this examination are given in Table V.

The use of absolute alcohol instead of rectified spirits for making up the 3 per cent. alcoholic soda affects the proportions of unsaponifiable material in the fat and the saponified extract. The high numbers obtained in previous examinations are probably due to the formation of resinous substances from the impure alcohol during the prolonged boiling necessary for the saponification. Since the quantity of unsaponifiable material is so much less, the percentage weights of normal and oxy-fatty acids is higher, because these percentages are calculated on the total weight of the three, e.g. forced rhubarb.

(1) TABLE IV.

Saponification with alcoholic KOH. (Methylated spirits used.)

Percentage by Weight.

(i) Unsaponifiable material $= 8.7$ grm. $\dfrac{8.7}{19.8} \times 100 = 43.9$

(ii) Normal fatty acids $= 3.0$,, $\dfrac{3.0}{19.8} \times 100 = 15.2$

(iii) Oxy-fatty acids $= 8.1$,, $\dfrac{8.1}{19.8} \times 100 = 40.9$

Total weight $= 19.8$,,

(2) TABLE V.

Saponification with alcoholic NaOH. (Abs. alcohol used.)

Percentage by weight.

(i) Unsaponifiable material $= 0.076$ grm.   2.1

(ii) Normal fatty acids . $= 1.09$,,   30.4

(iii) Oxy-fatty acids $= 2.42$,,   67.5

Total weight $= 3.586$,,

Since it is the comparative amounts of normal and oxy-fatty acids that are important and not the absolute amounts, in considering these results it is the ratio of one to the other that must be taken into account. Thus in Table III the ratio of normal fatty acids to oxy-fatty acids is :

		Normal.	*Oxy-acids.*
Rose	Fat =	1	1.9
,,	Sapd. Ext. =	1	93.0
Chrysanthemum	Fat =	1	0.35
,,	Sapd. Ext. =	1	16.0

In Table IV :

Forced rhubarb	Fat –	1	0.28
,, ,,	Sapd. Ext. =	1	2.7
Outdoor-grown	Fat =	1	0.7

In Table V :

			Normal.	Oxy-acids.
Forced rhubarb	Sapd. Ext. =	1		2·2
Outdoor-grown	,, ,, =	1		5·3

The flower petals have very high proportions of oxy-fatty acids, and in the comparisons of the forced and outdoor-grown specimens of rhubarb the outdoor-grown has always the higher proportion.

e.g.　Table IV　1 : 0·28　and　1 : 0·7
　　　Table V　1 : 2·2　and　1 : 5·3

In each case the outdoor-grown has about twice as much oxy-fatty acid as the corresponding quantity in the forced rhubarb.

IV. DISCUSSION.

From the results of these analyses, cutin is seen to be a complex mixture of fatty substances consisting of :

1. Free fatty acids and condensation products of fatty acids.
2. Fatty acids combined with alcohols.
3. Soaps.
4. Unsaponifiable material.
5. Carbohydrate basis.
6. Resinous substance and a compound giving tannin reactions.

A large amount of the fatty acids present are of the oxy-fatty acid type. This is to be expected since the acids have been exposed to the oxygen in the atmosphere for considerable lengths of time. The cuticle of the flower petals contains larger amounts of oxy-fatty acids than that from the petioles of forced rhubarb (the saponified extracts of rose petal cuticle containing over 90 per cent.; and chrysanthemum 80 per cent., while the forced rhubarb has only 40·9 per cent. and 67·5 per cent.). These oxy-acids are less soluble in chloroform than the normal fatty acids (the greater quantity of which are in the chloroform soluble fat), and are only extracted from the cuticle after a lengthy saponification. In the chrysanthemum, the fat contains 45 per cent. of normal fatty acids and 16 per cent. of oxy-fatty acids, while the saponified extract contains 5 per cent. of the normal fatty acids and 80 per cent. of oxy-fatty acids.

The nature of the substances present in the non-saponifiable material remains in doubt, although compounds of the nature of sterols are suspected. From the fat of the cuticle of chrysanthemum petals, a phyto-sterol-like compound was isolated which gave many of the colour reactions of this alcohol, and gave a crystalline acetate. The melting-point of this acetate was approximately 70° C., much too low for phytosterol acetate, but resembling some of the alcohols isolated by Fargher and Probert in their benzene extract of American cotton (5). Since no glycerol has been identified, it seems that the fatty deposit has the characteristics of a wax,

rather than a fat, and has, as its base, monohydric alcohols such as phytosterol rather than the trihydric alcohol, glycerol.

The high percentages of unsaponifiable material found in both the fat and the saponified extract cannot be attributed entirely to the cutin deposits. Some of it may be due to impurities from cell contents and colouring matter, but it is in this extract that higher alcohols, such as Fargher and Probert found, may be present, but so far these have not been isolated.

The percentage of ash present in cutin is very low, 2·35 being the highest number obtained. If all the ash obtained be assumed to be due to soap-forming metals, only a very small proportion of the fat can be present as soaps at the time when the cuticle was collected. A higher percentage than is apparent may have come to the surface as water-soluble soaps of fat and have been leached out by the rain. The relatively high ash value of the forced rhubarb cuticle, 2·35 per cent., as compared with the low value for outdoor-grown, 0·85 per cent., may partly be accounted for in this way, although the percentage of ash in the cuticle varies considerably in different species of plants.

The cutinogenic fatty acids remain soft and waxy even after several weeks' exposure to the light. It is only on heating to fairly high temperatures that a brittle varnish-like layer is produced and any appreciable decrease in their solubility takes place.

The importance of the large proportion of the residue left after the fat extraction and saponification of the cuticle has already been discussed in a previous paper (9). Oxidation and condensation in the fatty compounds seem to be accompanied by a drying process in the cellulose wall underlying the fat, which changes from the form of cellulose found in the cuticle of the young shoot, when it is easily hydrolysed by sulphuric acid, to some anhydride form that resists hydrolysis.

SUMMARY.

1. Cutin and suberin, although generally similar, show many differences, amongst these being the absence from cutin of phellonic acid and glycerol.

2. Cutin is a complex mixture of fatty acids, both free and combined with alcohols, that have undergone condensation and oxidation; soaps of fatty acids and unsaponifiable material which probably contains some higher alcohols; resinous substances and a compound giving tannin reactions.

3. The relative quantities of unsaponifiable material, oxy-fatty acids, and normal fatty acids vary, but there is always a higher total quantity of oxy-fatty acids than of normal fatty acids.

4. The preponderance of oxy-fatty acids is the result of oxidation processes taking place during the deposition of the cuticle.

Throughout this work, Professor Priestley has very generously given helpful advice and criticism, for which the writer would like to express her sincere thanks and appreciation.

During part of the time this work was in progress in the Botanical Department of the University of Leeds, the writer held a Scholarship from the Department of Scientific and Industrial Research, to whom and to the Royal Society for grants to cover the cost of collection and preparation of material she desires to express her acknowledgements.

Messrs. Rowntree of York by lending the large metal Soxhlet, and the firm of Messrs. Hirst, Brook & Hirst, of Leeds, by drying large quantities of material at low temperatures, have also greatly facilitated the handling of material on a comparatively large scale.

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The Relation between Water Content and Photosynthesis.

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With five Figures in the Text.

IN a previous contribution by the writer (3) it has been shown that the photosynthetic activity of a leaf does not terminate abruptly, but first ceases in the marginal and intravascular regions of the leaf and then gradually proceeds inwards towards the centre and the main veins. This characteristic decline in the photosynthetic activity of a leaf is explained as resulting from the limitations of water-supply. The shortage of water is not due to loss of function on the part of the water-conducting elements, but is caused by the inadequacy of the water-supply arising out of increased demands for it.

In order to obtain experimental evidence in support of this view previously advanced. and to show by quantitative measurements that the rate of assimilation falls as the water content becomes low, it is necessary to measure the rate of assimilation for each leaf separately and to correlate the photosynthetic activity of the leaf with its water content.

Various types of apparatus have been devised by different workers to measure the gaseous exchange in plants. Besides the ordinary gas analysis apparatus of Winkler (18), Hempel (6), and Haldane (5), determination of small quantities of oxygen and carbon dioxide have been made with a specially devised micro-eudiometer by Timiriazeff (14) and with the capillary eudiometer of Bonnier and Mangin (see Aubert (1) and Thoday (13)).

These eudiometric methods of measuring photosynthesis are open to two objections: (1) The composition of the gas in the leaf chamber is not constant during the course of an experiment, and (2) the water relations of

the leaf are liable to be disturbed. These objections are removed by the use of the continuous current method, which was first employed by Kreusler (7), and which has been subsequently modified by Giltay (4), Matthaei (8), Blackman and Matthaei (2), Brown and Escombe (2 a), Willstätter and Stoll (18), and Spoehr and McGee (12). The main principle of the continuous current method is that a constant stream of gas containing a known percentage of carbon dioxide is passed through the leaf chamber in which the assimilating plant material is contained. The percentage of carbon dioxide in gas leaving the leaf chamber is again determined. Different methods are adopted by different workers to measure the unabsorbed carbon dioxide. All the above-mentioned workers while determining the rate of photosynthesis in land plants, either eudiometrically or by the continuous current method, have used leaves detached from the plant for their experiments. This can be counted as an objection inasmuch as the assimilating leaf material is not under natural conditions, though the other external conditions are more or less so in some cases. A leaf detached from the plant is not probably in the same conditions of health and physiological activity as it is when it is on the plant itself. Though a severed leaf continues to be physiologically active and carries on its life processes, it cannot do so long on account of various internal causes, and its physiological activity soon begins to lose its intensity. Though the petiole of the leaf in such experiments is kept immersed in water, the conduction of water is not likely to be as perfect and vigorous as it is in an undetached leaf. The rate of assimilation also does not remain the same, as the accumulation of the photosynthetic products takes place where they are formed, which retards and ultimately stops further production unless they are translocated. The rate of diffusion of such photosynthetic material in water is very probably much slower than the rate of translocation under natural conditions, and some substances very possibly do not diffuse at all on account of the interference of the plasmatic membrane as well as the complex laws governing the diosmosis of substances.

Again, in all these experiments, after the leaf is severed from the plant, a certain amount of time is taken in fixing and sealing the leaf in the leaf chamber, and in other manipulations, before an actual measurement of the rate of assimilation is made. This intervening period is probably sufficiently long to alter the internal conditions of the leaf and make the determinations unreliable.

These methods of estimating the rate of assimilation of a plucked leaf are quite unsuitable for the purpose of this investigation as the water content of the leaf very probably continues to diminish during the course of experimentation. So it is considered essential to measure the rate of assimilation of a leaf while it is attached to the plant and to measure its water content after the assimilation value is obtained. McLean (10) has

measured assimilation of Palm leaflets which are attached to the plant, but evidently he has not worked under controlled conditions. To work with a leaf attached to the plant involves a fresh complication in the arrangement of the apparatus. In the experiments of the above-mentioned workers, the leaf that is experimented upon is kept under constant and natural conditions when its assimilation is measured, but in the case of a leaf on the plant the leaf, as well as the whole plant, should be kept under the same uniform conditions. The following apparatus was devised to meet this special requirement. It was also designed with a view to repeat the work of the early investigators on various phases of photosynthesis after the completion of the present investigation, as it would be very interesting to compare the results obtained with a plucked leaf with the results that might be obtained with a leaf attached to the plant.

DESCRIPTION OF THE APPARATUS.

Water Bath. A big tank made of galvanized iron was used as a water bath (Fig. 1, A). It was filled three-fourths with water and the temperature of the bath was regulated and kept constant by a toluol thermo-regulator. The water in the tank was kept in motion by a stirrer, s, to ensure uniform heating. The stirrer was worked by a water turbine (not shown in the figure).

Plant Chamber. A second tank made of the same material was used as a plant chamber. It was much smaller than the first and was kept immersed in water. In order to keep it steady and prevent it from floating, due to the upward pressure of water, fifty-six pounds of melted lead were poured on the bottom of the tank, both inside and outside. Thus it was made sufficiently heavy to remain steady. The air in the plant chamber was continuously sucked out by means of a long tube, which reached the bottom of the plant chamber, the other end of the tube being connected to a water suction-pump. The fresh air was allowed to enter the plant chamber through a small tube fixed near the edge of the chamber. By this device the carbon dioxide content of the air in the plant chamber was kept constant and was not allowed to diminish on account of its absorption by the plant during the course of an experiment.

Leaf Chamber. In the continuous current method the leaf chamber is generally kept under water, and so it is not a difficult matter to regulate its temperature and keep it constant. But while working on a leaf attached to the plant, the same method of keeping the leaf chamber under water cannot be employed. If the leaf chamber is not kept under water, its temperature continues to rise under the influence of the powerful lamp. To meet the difficulty a new type of leaf chamber was devised, which worked quite satisfactorily. A rectangular box of zinc, 7 in. by 6 in. by 2 in., was
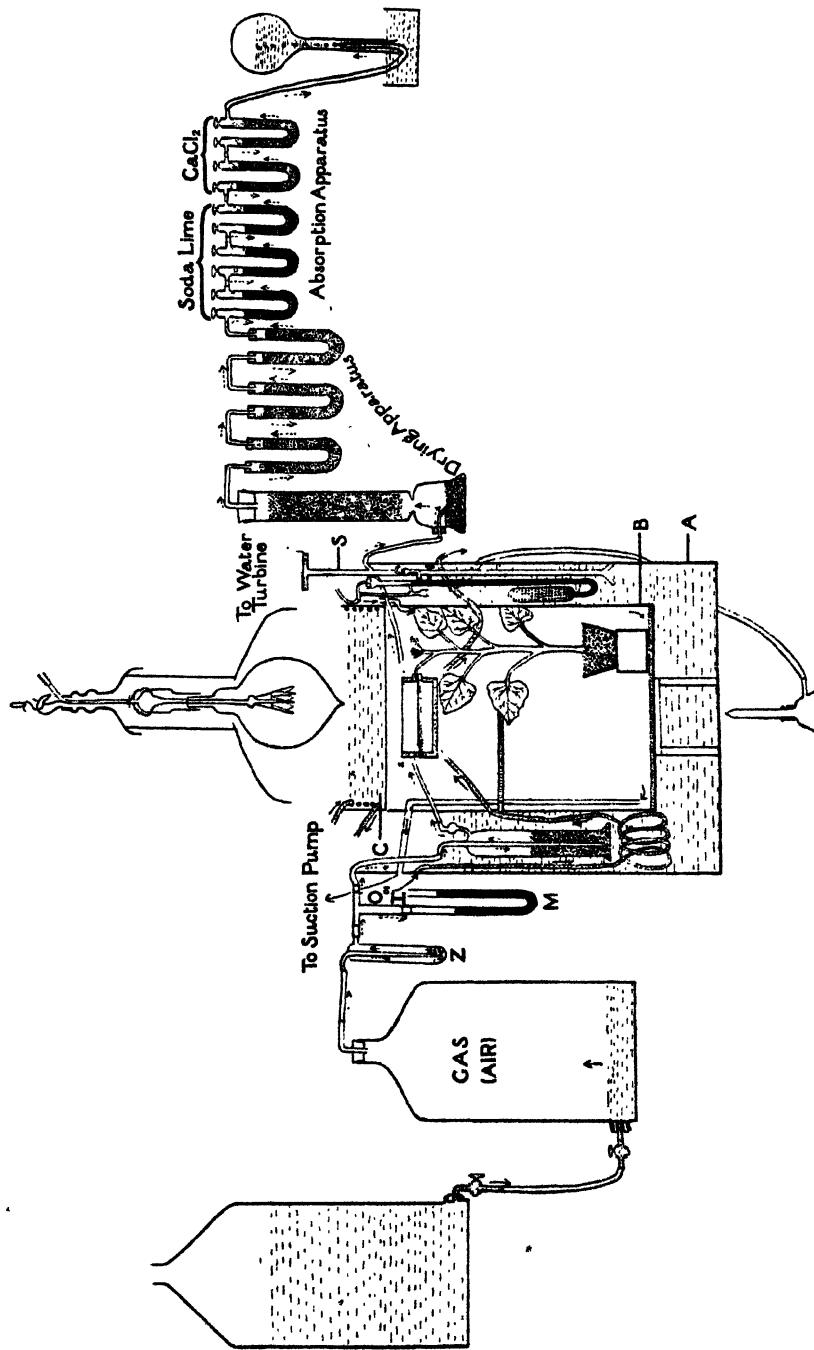
FIG. I. Diagram of the whole apparatus in longitudinal section. × ⅛. z = bubbler; M = manometer; s = stirrer; c = cooler; A = outer tank; B = plant chamber. (For explanation see the text.)

made with one of the two large surfaces made of glass, and it was surrounded on all sides by a water-jacket half an inch deep, except on the glass surface. Two holes with projecting tubes were made in·the water-jacket for the inlet and outlet of water. Similarly two tubes which passed through the water-jacket and opened in the interior of the leaf chamber conducted the gas stream. One of the two tubes, which was long, was for conducting the gas into the chamber, and the other, which was short, conducted the gas out of it. A big hole 1 in. across with a projecting tube $\frac{1}{2}$ in. long served for passing the leaf inside the chamber, and a smaller hole was made to take a thermometer (Fig. 2). A wire gauze of the same length and width as the leaf chamber was fixed inside in such a manner that it stood a little above
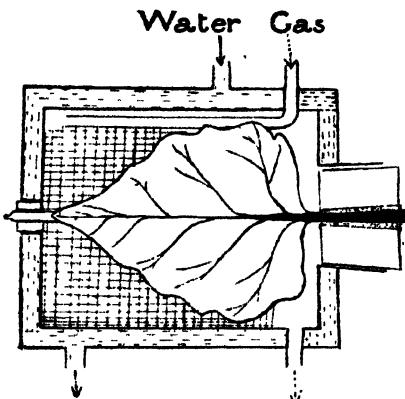
FIG. 2. The leaf chamber in horizontal section, showing the leaf, wire gauze, gas tubes, and the water-jacket. × $\frac{1}{3}$.

the bottom of the chamber. The leaf when enclosed inside the chamber rested on the wire gauze, and gaseous exchange on its under surface would not be obstructed. This would obviously be the case if the leaf was allowed to rest on the bottom of the chamber.

Temperature. The temperature of the plant chamber, i. e. the temperature of the air surrounding the plant, was regulated by the water bath. The temperature of the bath was raised and kept constant by the thermo-regulator at a certain degree which was sufficient to keep the plant chamber at a required temperature. In regulating the temperature, the cooling effect produced by the fresh air entering the plant chamber and the heating effect produced by the heat from the lamp had to be allowed for. The temperature of the plant chamber was indicated by a thermometer. A temperature of 20° C. was selected as a suitable one at which to measure assimilation ; it is an ordinary temperature in greenhouses and is not near the extremes of temperature under which some plants grow.

While measuring the rate of respiration in order to obtain the value for

real assimilation, the temperature of the water bath had to be raised, as the temperature of the plant chamber fell below 20° C. on account of the removal of the light.

The introduction of a water-jacket proved of great value in regulating the temperature of the leaf chamber. While measuring the assimilation cold water was made to circulate slowly inside the water-jacket to maintain the temperature at 20° C. When a leaf chamber without a water-jacket was used, the temperature inside it rose to 25° C. or more, depending upon the distance of the light from the chamber and the room temperature. By circulating cold water in a water-jacket the temperature could be lowered and kept constant at 20° C. If on a very cold day it fell below 20° C., the water circulating through the water-jacket was slightly warmed in a water heater before it entered the jacket.

In the same way during the measurement of respiration the water was warmed to maintain the same temperature. Thus the maintenance of the same and constant temperature in the leaf chamber and in the plant chamber required little manipulation.

Light. A 220-volt, 1,500-watt Mazda gas-filled lamp was used as a source of light, and it was kept at a distance of 35 cm. from the leaf in the leaf chamber. The heat from this powerful lamp was so great that it had to be absorbed to some extent by a cooler which was placed between the lamp and the leaf chamber. A circular dish of the same diameter as the plant chamber with a glass bottom and $2\frac{1}{2}$ in. high zinc wall was used as a cooler (Fig. 1, C). A layer of cold water 2 in. deep was kept in the cooler and was prevented from getting hot by means of the coils of lead tubing through which cold water was continuously circulated.

Blackman and Matthaei (2) have given the light intensity in their experiments in terms of candle-power at a certain distance, and the same has been given by Willstätter and Stoll (17) in the terms of Lux, *i.e.* the intensity of illumination produced on a rectangular surface by a Hefner candle at a distance of one metre. Though these data give a fairly good idea of the intensity of illumination used in their experiments, they do not convey any idea of the total radiant energy that was supplied to the leaves during the experiments, as a large amount of the radiant energy is absorbed in the water and in the glass.

The measurement of radiant energy in regard to problems connected with carbon assimilation has been attempted before by Detlefsen (3 *a*), Mayer (9), and Ursprung (15); Brown and Escombe (2 *a*) were the first to make an extensive series of such measurements, but the results obtained by them were not very reliable. Similar determinations have been made by Puriewitsch (11) and Warburg and Negelein (16) by means of a bolometer. The measurement of the total radiant energy in this investigation was made by a Moll's micro-thermopile supplied by the Cambridge Scientific

Instrument Company; it possesses a small heat capacity and high sensitivity.

In all the experiments in the present investigation, the same lamp was used, the distance between the lamp and the leaf was kept the same, and the layer of the water in the cooler was also of the same depth. So it was not difficult to have the same amount of total radiant energy arriving per sq. cm. per second in all cases.

Drying Apparatus. The gas issuing from the leaf chamber was rendered completely dry before it entered the absorption tubes. Pure strong sulphuric acid, with pumice stone and dry calcium chloride, was used to absorb the moisture from the gas. Fresh acid and calcium chloride were used for each set of six experiments and all traces of carbon dioxide were removed from the calcium chloride before the experiment. In order to remove all traces of moisture it was found necessary to pass the gas very slowly and to expose it to a large surface of the drying material. The results of many experiments had to be discarded on account of failure to obtain constant and consistent results due to incomplete drying of the gas. The presence of unabsorbed moisture leads to too high results.

Absorption Apparatus. The carbon dioxide in the stream of gas issuing from the leaf chamber has been determined in different ways by different workers. Since a very high percentage of carbon dioxide has often been used by them, the determination of the remaining quantity of carbon dioxide is not as difficult as it is when ordinary air is used. The air contains very minute quantities of carbon dioxide, and so in order to reduce the experimental error the absorption apparatus should be very perfect. Small differences which would come within the experimental error when working with high percentages of carbon dioxide would swell the percentage error to a great degree when ordinary air is used. After careful considerations of all the various methods of estimating carbon dioxide, the weighing method was regarded as the most reliable, as the experimental error tends to be very great in all the other methods. Moist soda lime was used for absorbing carbon dioxide, and calcium chloride for reabsorbing the moisture taken up by the dry gas as it passed through the moist soda lime. The total increase in the soda lime and calcium chloride tubes was taken as the weight of unabsorbed carbon dioxide in the volume of air that was passed through them.

The absorption tubes were weighed under the same conditions before and after the experiments. Slight changes in the temperature at the time of weighings made appreciable differences. The sides of the tubes were wiped thoroughly with chamois leather to remove the moisture adhering to them. Weighings were done as quickly as possible after each experiment. Each morning the tubes were weighed afresh before starting an experiment. All known precautions were taken to minimize the experimental

error. Soda lime and calcium chloride were renewed for work with each fresh leaf.

Gas. In order to obtain the values for assimilation as it takes place in nature, ordinary air was used for all experiments, though it is intended, in future, to obtain the assimilatory values with higher percentages of carbon dioxide. The air was enclosed in an aspirator bottle of eighteen litres capacity, and it was passed into the leaf chamber by allowing water to enter near the bottom from a big jar placed at a higher level than the aspirator. The gas (air) was first made to pass through a bubbler (z) containing water which helped in regulating the flow of the gas. The latter was then passed through a manometer which indicated the pressure at which the gas was flowing inside the apparatus. It was then conducted to a cylinder containing moist glass wool. Thus the gas, before it entered the leaf chamber, was rendered moist and did not lower the humidity of the air surrounding the leaf.

When the aspirator bottle became filled with water the same water was again put in the jar in order to avoid fresh absorption of carbon dioxide from the gas (air) in the aspirator.

Willstätter and Stoll (17) measured the volume of the gas passed through the absorption tubes by a gas-meter. A gas-meter gives an accurate measure of the volume of the gas when a very large volume is to be measured, but for small volumes of gas it does not yield reliable results. In order to dry the gas completely before it passed through the absorption apparatus the gas had to be passed very slowly, and the rate of flow of the gas was kept at one litre per two hours in all the experiments. This rate is sufficient for maintaining a steady supply of fresh air round the leaf.

The volume of the gas, as it leaves the absorption tubes, was measured by a measuring flask by the ordinary method of downward displacement of water. In all experiments the volume of the gas passed through the absorption apparatus was one litre. This volume was then corrected to normal temperature and pressure. The pressure as indicated by the manometer was added to the atmospheric pressure at the time of each experiment.

METHOD OF EXPERIMENT.

A leaf was carefully rolled without damaging the lamina or the small veins and was pushed through the big hole inside the leaf chamber until the whole leaf surface was right inside it. The leaf surface was then unrolled by a rod and the petiole of the leaf was held between the two halves of a rubber bung with one hole. The groove of the hole was lined with cotton-wool to avoid damage to the petiole. The rubber bung was then pushed into the hole of the chamber and was made completely air-

tight by packing plugs of cotton-wool all round the small gaps between the rubber bung and the tube of the hole and pouring in melted cocoa butter. The whole leaf chamber was thus made completely air-tight. While sealing a leaf the petiole is easily damaged, or sometimes is broken altogether, if slight pressure or pull is exerted on it. A fresh leaf was always enclosed if the petiole suffered the slightest injury during the process of sealing a leaf. While transferring the whole plant with the leaf chamber to the plant chamber in the water bath, the petiole of the leaf fixed in the leaf chamber should be guarded against injury on account of the same causes. The pot and the leaf chamber should be simultaneously lowered inside the plant chamber. If one is lowered more quickly than the other the weight of the leaf chamber breaks off the petiole or the petiole is bent and the inner woody tissues are crushed; with a little practice it is not difficult to manipulate the whole operation single handed.

The distance of the leaf from the lamp was adjusted before the plant was placed inside the plant chamber, as the distance varied according to the height of the plant and the distance of the leaf from the apex. So the pot required to be raised or lowered by blocks of wood, as the case might be, and to save the leaf from any injury the distance was fixed before the plant was put in the plant chamber.

The leaf chamber was then connected to the gas apparatus and the gas was allowed to flow through the chamber. Before connecting the leaf chamber to the drying apparatus test was made to ascertain that the leaf chamber was completely air-tight.

The water-jacket was afterwards connected to a water tap and the water was allowed to flow through it, and run out through the outlet. The cooler was placed on the top of the plant chamber and cold water was circulated through the coils of the lead tubing. The light was switched on, the leaf chamber was connected to the drying and absorption apparatus, and the gas was bubbled through the whole apparatus for a preliminary period of two hours each time a fresh leaf was enclosed. This was done to drive off all air from the leaf chamber and the drying and absorption apparatus, and to replace it with the air which had passed over the leaf and contained the carbon dioxide unabsorbed by the leaf. The air that passed through the absorption tubes when the experiment was started, and the volume of the gas measured, should have the same composition as the gas in the leaf chamber. This would not be the case if the gas were not allowed to bubble for a long time before an experiment was started. Such a procedure was necessary, as the gas leaving the leaf chamber could not be directly passed into the absorption tubes. It was necessary to let the gas flow for two hours under the following circumstances: (i) after a fresh leaf was enclosed; (ii) before an experiment to measure respiration was started after an experiment on assimilation;

(iii) before an experiment on assimilation was started after an experiment on respiration ; (iv) before an experiment was started eveiy morning if the leaf had been kept enclosed in the leaf chamber overnight. (A large quantity of carbon dioxide accumulates if the gas is not kept flowing overnight.)

The absorption tubes were then disconnected, though the gas continued to flow through the rest of the apparatus, and were weighed. The temperature of the room at the time of weighing was noted. The absorption tubes, during an experiment on assimilation, are slightly warmed on account of the heat from the lamp. So it is necessary to keep them in the balance before they are weighed. Similarly the absorption tubes should be reweighed if an experiment on respiration is started after an experiment on assimilation, as the last weight recorded would be the weight of the tubes after they were exposed to light, and therefore they require to be reweighed after keeping them in dark for some time. If these precautions are not taken the results are vitiated.

The experiment was started by reconnecting the absorption tubes to the drying apparatus and collecting the gas leaving the last absorption tube by displacement of water in an inverted one-litre flask over water. The flow of gas was regulated to one litre per two hours. If at the end of an experiment it was noticed that the time taken for one litre of gas to flow through the apparatus was more or less than two hours by more than five minutes the result of that experiment was discarded.

The absorption tubes were disconnected and reweighed under the same conditions as before. The total increase in weight of all the tubes was taken as the weight of carbon dioxide which was unabsorbed by the leaf from one litre of the gas.

In commencing an experiment to measure respiration the light was switched off, the cooler was removed, and the plant chamber was covered with a black cloth. The room was kept dark in all the experiments so that no sunlight reached the plant during the experiments on assimilation or respiration. The temperatures of the plant and leaf chambers were raised to 20° C., by methods described above, as the temperatures fell in both the chambers on the removal of light. The gas was allowed to bubble through for two hours and the same process was repeated as before. The total increase in weight of the absorption tubes was taken as the weight of carbon dioxide present in one litre of gas plus the weight of carbon dioxide evolved by the leaf in respiration.

If a is the increase in weight of the absorption tubes in the assimilation experiment,

and b is the increase in weight in the respiration experiment of the same leaf,

then, $b-a$ = real assimilation, for if c is the weight of carbon dioxide in the

same volume of the gas as is used in an assimilation or respiration experiment,

$c - a$ = weight of carbon dioxide absorbed by the leaf during assimilation in two hours,

$b - c$ = weight of carbon dioxide evolved by the leaf by respiration in the same period.

Therefore, $c - a + b - c = b - a$ = real assimilation.

With each leaf, three experiments on assimilation and three on respiration were performed if the results obtained were constant in each set of three experiments. The experiments were repeated in case the results varied.

The results of about the first fifty experiments were discarded, as they were complicated and not constant. This was traced to imperfect drying of the gas and many other minor defects.

But after the whole apparatus was once made perfect the results obtained were constant. Sometimes the result of any one of the three experiments on assimilation or respiration diverged beyond the experimental error from the results of the other two experiments. This appeared to be quite unavoidable on account of the minute quantities of carbon dioxide which had to be weighed. The weighings varied between 0·0007 grm. and 0·0025 grm. In the case of a divergent result more experiments were performed till constant values were obtained. No results have been discarded after constant values had once been obtained.

The figures given on p. 780 for each leaf are the sum of the mean of three results for both assimilation and respiration experiments.

After the value of real assimilation for a leaf had been determined, the leaf was carefully removed from the leaf chamber and was examined to see if it was in any way damaged during the period of experimentation; in all cases the leaves were found in perfect condition. The area of each leaf and its wet weight were then immediately recorded, and the dry weight of the leaf was determined after drying it at 100° C.

Plants with large leaves were selected for experiments, and they were kept in a greenhouse at 20° C. before they were worked with.

It is interesting to note that the different varieties of *Cineraria stellata* have leaves with different water content and different rates of assimilation. The last two varieties of the species were not in flower when these experiments were carried out, and so it was not possible to work with more leaves of the same varieties on account of the difficulty of identification from such a large number of varieties grown in the gardens.

The experiments were started in August and completed in December.

Wet Weight of the Leaf.	Dry Weight of the Leaf.	Total Leaf Area in sq. cm.	Water Content per 100 sq. cm. of the Leaf Area in grm.	CO_2 assimilation per 100 sq. cm. of the Leaf Area in grm.
		Abutilon Darwini, Hook.		
1·9150	0·3380	142	1·110	0·00130
1·7110	0·3420	122	1·122	0·00155
1·6810	0·3475	126	1·058	0·00043
1·5530	0·3410	115	1·053	0·00034
1·4970	0·3320	110	1·043	0·00018
		Spermannia africana, L.		
3·3140	0·6620	144	1·840	0·00132
2·8550	0·6845	125	1·730	0·00110
3·3650	0·7190	156	1·700	0·00102
3·2450	0·6520	156	1·660	0·00092
3·1230	0·7810	147	1·590	0·00080
3·0540	0·7940	151	1·490	0·00054
3·9060	0·9340	202	1·470	0·00049
		Cineraria stellata, var. *hort.*		
5·3010	0·3390	167	2·970	0·00083
6·1580	0·4160	213	2·890	0·00075
6·0010	0·3980	196	2·850	0·00070
6·6100	0·3780	184	2·840	0·00068
4·8450	0·3550	176	2·550	0·00045
		Cineraria stellata, var. *hort.*		
3·3270	0·2250	147	2·110	0·00088
3·4340	0·2590	151	2·100	0·00079
3·5610	0·2740	160	2·050	0·00050
		Cineraria stellata, var. *hort.*		
3·8940	0·2140	140	2·620	0·00078
4·6840	0·2500	172	2·570	0·00075
4·1000	0·2380	158	2·440	0·00063

DISCUSSION.

The results obtained indicate a close relationship between the rate of assimilation and the water content of a leaf. With decrease in rate of assimilation there is a corresponding fall in the water content of the leaf.

The relations between the two are so close that when the rates of assimilation of the leaves of any plant are plotted against the corresponding values of the water content, the points lie very nearly on a straight line. This may be regarded as a straight line having regard to the various sources of error (Figs. 3, 4, and 5).

The water content per unit area of the leaf differs in different plants, and, as has been observed in *Cineraria stellata*, it is also different in different varieties of the same species. The decrease in the rate of assimilation is

not the same with the same decrease in the water content in all the plants. Each plant has its own rate of decrease in the water content with the same decrease in the rate of assimilation.

The leaves of *Abutilon Darwini*, Hook., have a greater rate of assimilation per unit area than those of the other plants examined, though the water content is less than what it is in the leaves of the latter. Consequently
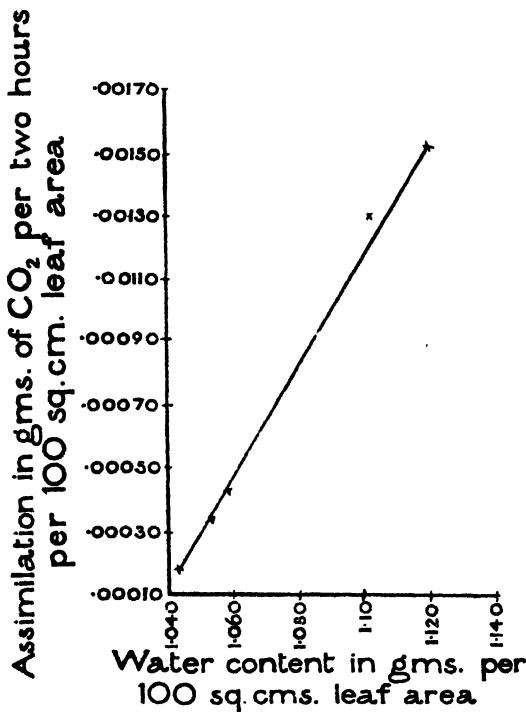
FIG. 3. Curve illustrating the relation between assimilation and water content in the leaves of *Abutilon Darwini*, Hook.

the decrease in the rate of assimilation is great for a small fall in the water content. Similarly the leaves of *Spermannia africana*, L., assimilate more vigorously than the leaves of the three varieties of *Cineraria stellata, hort.*, though the water content is less in the leaves of the former than in those of the latter. The three different varieties of *Cineraria stellata, hort.*, obey the same rule.

Three important facts are noticed on examining the results. (1) The decrease in assimilation of leaves is accompanied by a decrease in water content which is different for different plants. (2) The leaves with a low water content assimilate more rapidly than the leaves with a high water

content.   (3) The fall in assimilation is great in the leaves with low water content for a small decrease of the latter.

The characteristic marginal and intravascular photosynthetic decay that has been observed in the leaves of various plants (3) suggested the possibility of a decline in the water-supply, which was first felt, as would be naturally the case, in the regions of the leaves situated farthest from the
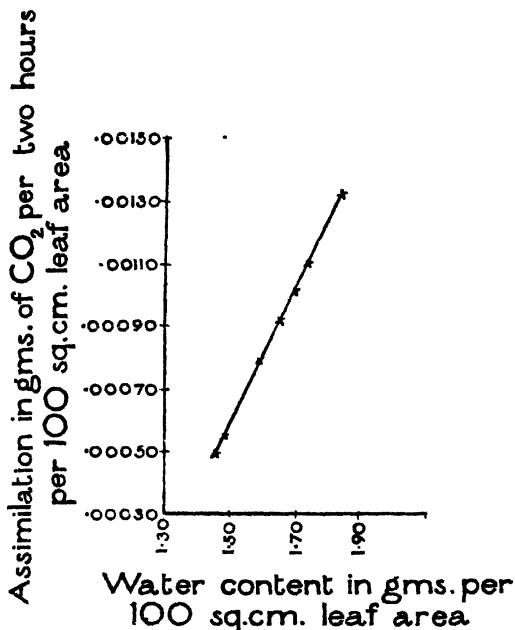
FIG. 4.   Curve illustrating the relation between assimilation and water content in the leaves of *Spermannia africana*, L.

water-conducting elements.   It has been suggested that the cause of the decline in the water-supply is to be traced to the increased demand for water as a plant grows and the total leaf area increases.   At a certain period the demand is in excess of the supply, and it causes the decay of some of the assimilating cells.

The results of this investigation support the above views advanced in a previous contribution (3) and clearly show that the water content of a leaf is directly related to its rate of assimilation.   However, it is not here maintained that the water content of a leaf is the chief controlling factor of assimilation, but it is hoped to arrive at a definite conclusion on this matter after further work.   Still, from the phenomenon of the marginal and intravascular decay of the leaves and the quantitative results obtained, it can be

concluded that the shortage of water eventually terminates the photosynthetic activity of a leaf, and consequently its duration.

Crop physiology is now receiving the attention of plant physiologists, and extensive investigations are being carried out on the effect on growth of plants of climatic factors, as well as the physical and chemical properties of the soil; and it is discovered that optimum external conditions and the presence of certain chemical substances in the soil promote the healthy growth of plants. But even under ideal conditions of growth a limit is reached when further improvement in any direction in plants is not possible,
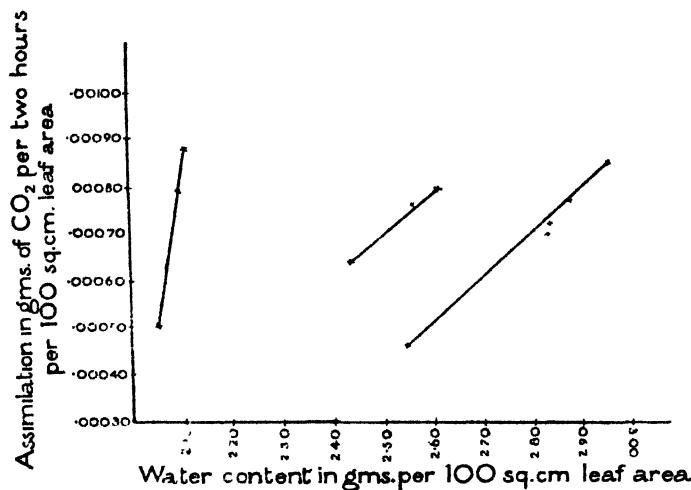
Fig. 5. Curve illustrating the relation between assimilation and water content in the leaves of the three varieties of *Cineraria stellata*.

and the failure to go beyond a certain maximum is attributed to internal and genetic factors over which we have very little control.

According to this investigation one of the internal factors limiting the growth and duration of life of plants appears to be in their conducting systems, which are not efficient to supply uninterruptedly the watery solutions of substances to the chief manufacturing organs of food of plants. Under ideal conditions the supply of essential elements does not run short, but such ideal external conditions do not increase the conductivity of the woody elements and save the photosynthetic cells from decay when the demand becomes greater than the supply inside a plant. As a result the photosynthetic elements perish and cannot be revived. Such periods of shortage terminate slowly the photosynthetic activity of a leaf, effects of which are first felt by the lower and older leaves on account of the two main causes mentioned in the previous paper (3).

It is also reasonable to assume that the absorption of water with dissolved substances is not rapid enough to meet the increased demands as the plant grows and the total leaf area increases, though it is known that fresh absorbing organs continue to be formed in increasing numbers during the duration of life of the plant. But the inadequacy of the supply of the watery solutions is not solely due to its inadequate absorption, as the formation of secondary conducting elements points to the necessity of increasing the area of the cross-section of the conducting tissue as a plant grows.

Similarly this area is also increased by the formation of larger woody elements in the spring than those formed in the autumn, as the demand for watery solutions is greater in the spring than it is in the autumn. Finally, the formation of new conducting elements does not take place in a fully grown leaf, and so the total conduction of the watery solutions remains the same even when the need for it increases. So the conclusion that the conducting system may not be efficient cannot be avoided.

If the rates of assimilation, transpiration, and absorption and the total water content are determined for a plant in different stages of development under natural conditions. it will be possible to obtain some facts regarding the demand and supply of water ; and if the facts thus obtained support the conclusion that is arrived at here, they will lead to new fields of physiological investigation.

I have to thank Dr. W. Stiles for the facilities given to me in his laboratory, and for criticism and references to literature during the course of the present investigation.

SUMMARY.

1. A modified form of apparatus devised for use with the continuous current method to determine the rate of assimilation of a leaf is described. The leaf is not detached from the plant during the experiment as has been done in the past. The leaf experimented upon, and the plant, are both kept under the same and constant conditions. A new type of leaf chamber has been specially devised for the purpose.

2. The measurement of the total radiant energy per sq. cm. per second arriving from a 1,500-watt, 230-volt, gas-filled electric bulb is made by a Moll's micro-thermopile. The measurement is made so as to give exactly the radiant energy in calories per sq. cm. per second that is supplied to the leaf, as a large amount of the energy is absorbed by the water in the cooler.

3. The results of the investigation show that (1) there is a distinct correlation between the decrease in the rate of assimilation and the fall in the water content per unit of leaf area, and when the two values for a set of

leaves of a plant are plotted, the points lie very nearly on a straight line ; (2) the leaves with low water content assimilate more rapidly than the leaves with high water content ; (3) the ratios between the decrease in assimilation and the decrease in the water content for different plants are different.

4. It is held that the decrease in assimilation may be due to the decrease in the water content caused by the shortage of water. The shortage of water occurs when the demand for it exceeds the supply as a plant grows. It is here suggested that the conducting system is not able to discharge its function vigorously enough to meet the increasing demands.

5. The inefficiency of the conducting system may probably be one of the internal limiting factors which terminate the duration of life of leaves, and ultimately of plants.

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On the Origin of the Cystocarp in the Genus Gracilaria.

BY

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With eighteen Figures in the Text.

I HAVE always felt, in the common occurrence of finely fruiting specimens of *Gracilaria confervoides* on the beaches of Anglesey and Carnarvon-shire, a challenge to examine the plant once more, in order to see if it were not possible to carry the story of the cystocarp a stage farther back than had been done by Thuret and Bornet in the 'Études phycologiques' (1878), or even by Johnson at a later date in this Journal (1887).

It may be recalled that, although Thuret and Bornet added greatly to our knowledge of the plant, they had to confess that they had never succeeded in finding the procarps. Johnson set out to fill up this gap, and undoubtedly described and figured some earlier stages in the development of the cystocarp than had been seen by the previous investigators. Yet it cannot be said that his account was altogether satisfactory.

My own hesitation about accepting Johnson's results in their entirety arose from a suspicion that the procarps probably arose on the thallus before any sign of the cystocarpial swelling appeared, and not, as Johnson seemed to find, within the swollen protuberance *after* it had begun to appear. I felt that the swelling, however rudimentary, was more likely to be a consequence of fertilization than a preparation for it. Johnson was fully aware of this objection, but regarded *Gracilaria* as a specialized case among Florideae, and compares it with *Chara* among Chlorophyceae, where also the female cell is, almost at its very inception, already enclosed.

Further, all analogy seemed to indicate that the procarps were probably much more numerous than the demonstrable swellings, mature and imma-ture, abundant though they are in *Gr. confervoides*. For example, in

Delesseria sanguinea there is never found more than one mature cystocarp on each fertile leaflet, although scores of procarps are produced (Phillips (1898)).

Oltmanns, in 'Die Algen' (1904), seems also somewhat dissatisfied with Johnson's account of the details of the development. He does not, it is true, raise the issue referred to above. But writing of the development of the fruit in Sphaerococcaceae (in which family *Gracilaria* at present falls), he refers to Johnson's 'unhappily somewhat incomplete statements' about *Gr. confervoides.* He points out in particular that Johnson had not made quite clear the structure of the carpogonial branch, nor the fusion of the carpogonium with the auxiliary cell.

To test my own doubts, and yet not quite cover the same ground as earlier investigators, I determined, in the first instance, to examine another species of *Gracilaria* altogether, namely *Gr. multipartita.* I observed that this plant was always included in the lists of algae procurable from the Laboratory of the Marine Biological Association at Plymouth, where it is, I believe, brought up with the dredge. It is not reported from the shores of Anglesey and Carnarvonshire, so that I have not been able to examine it in the fresh condition. There was also the further disadvantage in the study of this species, as compared with *Gr. confervoides*, that male and tetrasporiferous plants were not available, as they had not apparently up to the time of publication of Holmes and Batters's 'List of British Algae' (1892) been reported from any station. Still, it is undoubtedly a species of *Gracilaria* with similarities to *Gr. confervoides* both in its minute anatomy and in the structure of its mature cystocarps. According to Harvey (1859) (who, by the way, has some description of the tetraspores, a fact overlooked by Holmes and Batters (1892)), some specimens of *Gr. multipartita* found in American waters approach *Gr. confervoides* very nearly. Judging from the material supplied to me from Plymouth, the two species seem distinct beyond doubt, yet the fact that they do approach one another in some forms makes it the more likely that they are closely related, and that conclusions drawn from the study of the one would be found to be applicable to the other.

1. *Gracilaria multipartita*, J. Ag.

This plant exhibits Oltmanns's 'Springbrunnen' type of apical growth, i. e. is devoid of one principal thread of cells, from which all others constituting the thallus are ultimately derived, but possessed instead of several leading threads growing in co-ordination, and spreading out like a fountain from behind the apex to build up the thallus. The structure is of course essentially filamentous throughout, with purely apical growth of the filaments, though the filamentous character is much disguised in the interior of the thallus.

The appearance presented in cross or longitudinal section may be gathered from some of the sketches accompanying this paper (Figs. 2 and 5). There is a core of large cells passing fairly abruptly into a surface layer of relatively small cells. There is, indeed, a narrow band of cells of intermediate size, but it seems in some places wanting, and is never more than one-celled.

The hemispherical cystocarps obviously arise in acropetal succession, though not strictly so, and are irregularly distributed in considerable numbers over the flattened surface, and even the edge of the thallus. Sometimes the cystocarps are crowded closely together; at others they are more sparsely scattered, even leaving in some places wide areas free from cystocarps altogether. This distribution seemed to suggest irregularities in fertilization rather than irregularity in the production of procarps. I accordingly examined carefully the surface of the thallus lying between the maturing cystocarps.

A procarp in the Florideae ordinarily consists of a specialized carpogonial branch ending in the carpogonium carrying a trichogyne, and one or more specialized auxiliary cells recognizable in some groups even before fertilization.

In the quest for procarps, I was therefore particularly intent upon looking for hairlike outgrowths arising on the surface, which might suggest a trichogyne.

Contrary to my expectation, for the plant looks to the eye completely glabrous, I found that unicellular hairs do undoubtedly occur in this plant, but are distributed sparsely and apparently at haphazard over small areas of the surface. It is difficult to see them on the bleached thallus, and not easy to find them in sections. I have found that a ready means of demonstrating their occurrence is to immerse the bleached thallus in a dilute solution of haematoxylin, when the hairs take up the dye with avidity. The cells from which they arise are somewhat larger than the ordinary surface cells, and stain slightly more deeply.

But these hairs have not the characteristics of trichogynes. The walls are not gelatinous, and show when mature a sharp double contour to the wall, while the lumen contains a minimum of granular matter, and, as I have said, they stain readily with haematoxylin, which, on account of the gelatinous wall, a trichogyne ought not to do, at any rate to the same degree.

I did find, however, lying among the superficial cells, some curiously large cells, often many scores to the square millimetre (Fig. 1). When the bleached material is stained in Hoffmann's blue, they are a conspicuous feature in the superficial layer, inasmuch as they absorb the stain with great intensity as compared with the smaller surface cells among which they lie. But although in such stained material they stand out among the

other superficial cells, I could not observe in them well-defined chromato-
phores such as occur in the ordinary surface cells, and would therefore
expect that in fresh material they would be conspicuous, not by their
deeper colour, but by being devoid of colour. I have figured them from
the surface view, when they appear elliptical in outline, the long axis
corresponding to that of the thallus, and again in sections (Figs. 2, 3, 4) cut
vertical to the surface in profile, when they appear ovate with the apex
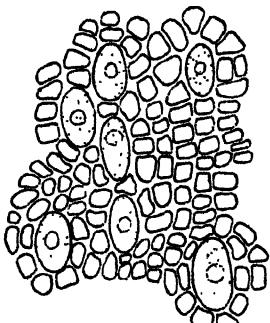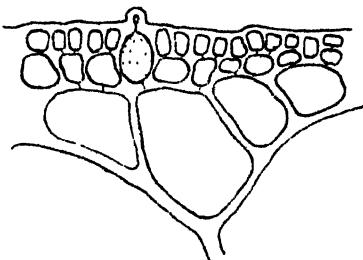
FIG. 1.   FIG. 2.

FIG. 3.   FIG. 4.

FIGS. 1–4. *Gr. multipartita.* Fig. 1. Surface view of carpogonia lying among the superficial
cells, with indications of papillae. Fig. 2. Vertical section showing papillated carpogonium, the
papilla swollen in dilute glycerine. Figs. 3 and 4. Similar views of carpogonia with collapsed
papillae.

directed outwards.   They do not protrude appreciably beyond the ordinary
surface, but extend downwards so as to lie among the intermediate cells,
with which they show pit-connexions (Fig. 2).   Occasionally one may be
seen lying directly attached to a cell of the core.   The deeper staining
of these cells is due to the fact that they are devoid of vacuoles and filled
with a granular protoplasm throughout.

I was inclined to think at first that these cells were mucilage glands, until,
upon closer examination and with higher magnification, they all presented
a curious feature which arrested my attention.   In tangential sections
I could commonly see defined upon their outer surface a small circular
disc-like area, which seemed to indicate the presence of a raised papilla
(Fig. 1).   In vertical sections this papilla could also be detected, and lying
within it a slight horizontal slit in the wall with a fine pit-like process passing
down from it to the apex of the ovate protoplast.   It was not an uncommon
occurrence to find that, whether viewed from the surface or in profile, fine

granules of foreign matter adhered to the surface of the papilla in greater proportion than elsewhere, suggestive of a more mucilaginous consistency of the wall at that spot. On warming vertical sections in glycerine, I was able to swell up the papillar process so that it assumed the appearance of a pronounced protrusion with granular contents communicating by a short thread with the contents of the cell below (Fig. 2). In this condition they reminded me strongly of Berthold's (1882) figures of the procarps of *Porphyra*, and I then conceived the idea that the large cells were the carpogonia of this plant, and the papillae the rudimentary trichogynes. It may be pointed out that the emergent portion of the trichogyne in many Florideae with a flat thallus such as *Nitophyllum* and *Delesseria* (Phillips (1898)) is hardly more than a papilla. The long, attenuated trichogyne is rather a characteristic of filamentous forms such as *Callithamnion.*

Following up the conviction that the large cells were derelict carpogonia without a carpogonial branch, and the papillae reduced trichogynes, I concluded that I ought to find them in a receptive condition somewhat in front of the youngest recognizable cystocarps immediately behind the growing-points.

Within a millimetre of the apex I found these large cells being differentiated among the cells of the surface, and behind that region they had already acquired the full size, granularity, and staining properties characteristic of them in the older parts. There, however, they were circular in outline when viewed from above, showing that their elliptical outline farther back is due to the elongation accompanying growth in length. I do not think that they are formed anew outside the apical area, where they seem more numerous per unit of area than in the older parts, owing to their being carried apart from one another by a general extension of the surface.

About five millimetres behind the apex, I found the first rudiment of a cystocarp, detectable by the slightly deeper colour of the material when stained in bulk. It follows that fertilization may occur considerably within this distance of the apex, and I believe it generally does. The carpogonia may remain receptive of a spermatium some distance farther back, but those I found among the maturer cystocarps were certainly derelicts and functionless.

I had hoped to find in this apical region papillae in better condition, since here they ought to be in the receptive stage. While, however, every carpogonium showed the same evidence of the existence of a papilla, the apex did not exhibit a continuous rounded outline in profile, but rather a flattened apex on the protuberant base, or even a depressed crater-like apex on the raised base (Figs. 3, 4). This may be due to collapse resulting from the method of fixation, and I certainly would expect to find a rounded protrusion in the fresh material. The gap in the wall within the protrusion,

and the pit passing down to the pointed apex of the protoplasm of the carpogonium, could be made out in all suitably sectioned material.

Assuming now that a fertilized carpogonium is the point of initiation of a cystocarp, what modification of growth takes place in the thallus at that point ? It would seem that the fertilized carpogonium immediately communicates a stimulus to all the superficial cells in the neighbourhood, the nearest first, and gradually those farther off, so that they become the apical cells of a crop of filaments growing closely packed together vertically to the surface. The first effect is that the fertilized carpogonium is overtopped and disappears below the raised surface, and then gradually all the unfertilized carpogonia around, as far as the stimulus to growth has extended. When the longest filaments lying immediately round and above the fertilized carpogonium have become about ten cells long, the young cystocarp is capable of detection from the surface by the deeper staining of the circular area affected. I have avoided speaking of the fertilized carpogonium as submerged, because it does not change its position relative to the intermediate cells and those of the core in this so-called submergence. It is rather that it is overtopped by the luxuriant growth of the neighbouring surface-cells. Its case is like that of a lost golf-ball in a rapidly growing sward. It remains precisely where it lay when first lost, but later seems submerged below the surface of the grass. This point is of some importance when it comes to the identification of the fertilized carpogonium within the swollen mass.

It might be supposed that in this overtopping process a pit or chimney would be left vertically over the carpogonium. While the surrounding surface cells are cutting off their first two or three cells, no doubt this is so, but the filaments soon overarch it, and abutting against one another grow vertically forward, leaving no gap. The cuticle, which is being raised unbroken, however, carries with it in a slight depression the external mark of the papilla and the remains of the spermatium attached thereto. In two cases only, however, have I been able to observe what I took to be the remains of a spermatium *in situ* (Fig. 5).

To trace the story of the zygote, after it has been embedded within the growing cystocarp, recourse must be had to vertical sections through the swelling, and tangential sections carried along the level of the general surface outside the swelling, by paring off the rounded outgrowth above it. From what has been said above, tangential sections taken at this level ought to pass through the submerged carpogonium, and experiment shows that they do.

Traced by this means, the zygote is found to continue to exert the same stimulus to growth which it does at the first, and the tubercle continues to extend at the circumference, and to increase in height towards the centre for a considerable time. Its most remarkable effect is, however,

upon the basal cells of those filaments which lie immediately round it. It greatly increases in bulk, and finds room by pushing them back all round (Figs. 5, 6). It is beginning, moreover, a course of parasitism which does not cease until the carposporophyte is mature. The stimulus which at first gave rise in the surface cells to the production of filaments brings about hypertrophic growth in all the cells near it belonging to the intermediate layer, and also in the basal cells of the filaments which arise from them. Upon these latter the zygote soon begins to prey. At first swollen, some of them obviously soon shrink in size to increase the bulk of the zygote. It then begins to send out pseudopodia among them and gradually incorporates them into its own substance. Its appearance at this stage is
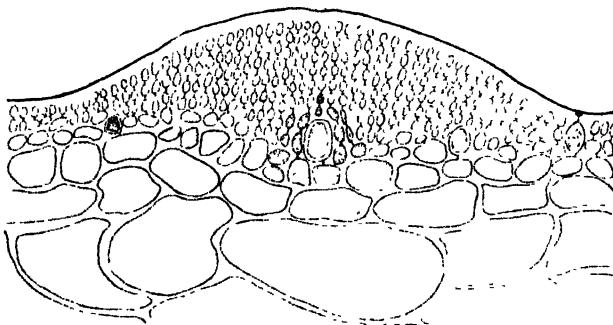
FIG. 5. *Gr. multipartita.* Median vertical section through a young cystocarp, showing a carpogonium at the surface just within the hypertrophied region, and three overgrown. The middle one is beginning to affect the neighbouring cells, indicated by the dotting in the drawing, and by the deeper staining in the preparation. A spermatium seems attached to the cuticle at the surface.

remarkable, being a giant cell with many tentacles lying like a great amoeba among cells, some of which it becomes confluent with, and others of which it exhausts of all their substance, either by a process of absorption through the walls, or by means of fine pits (Figs. 5. 6, 7, 8, 9).

I cannot find that there is any specialized auxiliary cell worthy of the name. The absorption and incorporation together involve the destruction of many scores of cells. The zygote may be called a fusion cell, or, to use Church's term (1919), a trophocyte. and there is no doubt that at this stage it has become multinucleate (Figs. 8, 9) presumably by the division of its own diploid nucleus.

The next stage in the development of the fusion mass, after it has foraged sufficiently on the neighbouring cells, seems to be the outgrowth of rounded protrusions upwards, outwards, and even slightly downwards. Each protrusion then seems to divide up ultimately into clumps of cells by the formation of cell-walls, and these cells become the apical cells of the prolific branch system of the new carposporophyte (Fig. 10). This causes a further absorption of the lower parts of the vertical threads, and a pressing

outwards and downwards of those which are not so absorbed. The power of solution and absorption would seem to remain with all the cells of the carposporophyte wherever they come into contact with the tissue of the gametophyte, but there is no further fusion similar to that which takes place in the earlier stage of development of the zygote, and the tissues of the gametophytic host and the parasitic carposporophyte remain distinct even at the flattened base, where the pressure of the parasite on the host is
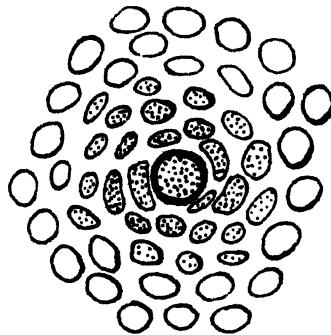
FIG. 6. FIG. 7.

FIGS. 6 and 7. *Gr. multipartita.* Fig. 6. Vertical section through young cystocarp, showing zygote beginning to send out processes among adjacent cells. Fig. 7. Tangential section through still younger zygote, showing region affected (dotted cells) more highly magnified.
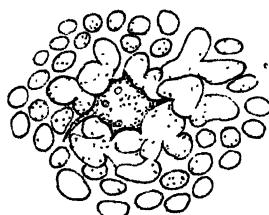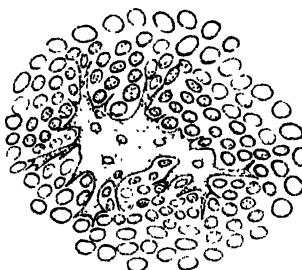
FIG. 8. FIG. 9.

FIGS. 8 and 9. *Gr. multipartita.* Fig. 8. Showing fusion of the zygote with basal cells of vertical filaments. Fig. 9. The same zygote higher up, showing extensive pseudopodial processes among the degenerated cells of the filaments. All the affected cells in both figs. dotted.

greatest. The existence of a single cell at the situation of the fusion cell can be demonstrated in all median sections cut vertically through the carposporophyte, and all the tissues of the carposporophyte are certainly derived from it (Fig. 1c). Every branch of the dense mass of the carposporophyte radiates from this cell, the same tale being told by tangential sections as well as vertical. I have observed, in some sections treated with iodine, that the cells of the host and parasite take on a different tinge of brown, showing thus a sharp delimitation of the tissues even where they are in closest contact.

To return now to the growth of the pericarp, as it may be called, to accommodate the growing carposporophyte. I have described how the zygote eats from the first into the bases of the vertical threads which lie immediately around it (Fig. 8). The stimulus to growth emanating from the zygote continues, and a widening circular area of surface gives rise to vertical threads until the limits of the mature cystocarp are reached, and the bases of more and more of the vertical filaments are dissolved away for the accommodation and nutrition of the growing parasite. How then is the nutrition of the remaining undissolved cells of what may be called the canopy of the tubercle provided for? It is by means of a copious system of secondary lateral pits (cross-pitting) which keep it still in communication with the body of the thallus by way of the flanks, though cut off from the base. Still, the canopy is at its weakest at the highest point, and it is here that it ultimately gives way to the upward push from below and the stomium is formed. The mechanical thrust of the growing carposporophyte is probably accompanied by the secretion of ferments which dissolve both the polysaccharide walls and the proteid contents. A clear gelatinous interval is thus formed at the area of contact of parasite and host, where the debris of the dissolving cells may be seen lying in the gelatin (Fig. 10).

FIG. 10. *Gr. multi-fartita.* Vertical section of cystocarp at the stage when cells are being budded off from the fusion-cell, to grow into the young carposporo-phyte.

The carposporophyte is at first more or less spherical in shape, then hemispherical, but as the thrust at the apex begins to prepare the way for the formation of a stomium, it tends to become conical. When it has matured sufficiently to give rise to the carpospores, they are formed all over the curved surface, and even from the flattened lower surface some distance beyond the edge of the cone. The differentiation of its tissues at this stage is curiously like that of the gametophyte upon which it arises. There is the same core of large cells, which has been called elsewhere the placenta, passing into an intermediate layer of smaller cells, which again bear the short chains of spores. It is the superficial layer which gives rise to the reproductive cells in both generations.

Before leaving the anatomy of the carposporophyte I might refer to some curious features that presented themselves in the course of the investigation. The first was the appearance of strong threads crossing over the clear space separating the carposporophyte from the canopy, and then growing irregularly among its vertical threads. I thought at first they might be a parasitic alga, but as I found them in organic continuity with the placenta, I concluded they must be an extra means of preying upon the tissues of the gametophyte, resorted to in the later stages of development of the cystocarp.

A parasitic alga does, however, undoubtedly attack this *Gracilaria*. In some cases, its attack is so severe as to bring about the arrest of the growth of the sporophyte. Iron-haemotoxylin treatment of microtome sections cut in paraffin blackens the parasite and picks it out to the eye. I have not seen any signs of its reproductive organs.

Another feature worthy of note is the appearance of the chromatophores of the carpospores as these mature. They are in shape short rods, and are bunched up in the middle of the spore, apparently round the nucleus, and look very like the stellate arrangement of chromosomes observed during mitosis. Similar stellate groups of chromatophores may also be found in cells of the core immediately beneath the sporophyte attachment. Iron-haematoxylin treatment brings out this appearance also.

To sum up here the results of the investigation of this plant. I came to the study expecting to find a carpogonial branch, ending in a carpogonium prolonged into a trichogyne, as the tradition requires. 'The female sexual cell', says Schmitz (1893), 'arises without exception out of the end-cells of shorter or longer branches.' The carpogonium is here simply an end-cell of an ordinary thread contributing to form the surface layer, of which it seems to be merely a specialized cell. It may rest on an intermediate cell, or directly on the larger core-cell. There is certainly no specialized carpogonial branch. Again, there is no elongated trichogyne of the type found in some Florideae. The receptive organ is more like that of the Bangiaceae. At a later stage I looked for an auxiliary cell. The fertilized carpogonium enters into fusion with a considerable number of adjacent cells, and absorbs the contents of hundreds of cells from first to last, but there are no such specialized cells, certainly no such prepared beforehand, as have been designated auxiliary cells in other Florideae.

2. *Gracilaria confervoides*, Grev.

This plant is reported by Holmes and Batters (1892) from all their fourteen sections of the British coast. The three other species, *Gr. multipartita*, *Gr. divergens*, and *Gr. compressa*, are all south of England species. If, however, Thuret and Bornet's (1878) dictum that the occurrence of the antheridia in crypts is a specific characteristic of *Gr. confervoides*, all the forms growing on the coast of Anglesey are not true *Gr. confervoides*. One in particular, found in rock-pools near high-water mark, has antheridia dispersed over an uneven surface, and has an upright bushy habit. The greatly elongated plant (Batters's var. *procerrima*), almost unbranched, also occurs. Even if, as I suspect, the *Gr. confervoides* of these parts is an aggregate of *petites espèces*, yet the tubercles are all so similar that there is not likely to be any difference in the mode of origin.

Gr. confervoides is credited by Church (1919) with having all three

kinds of reproductive structures, tetraspores, antheridia, and cystocarps, occasionally on the same plant. I have never seen tetraspores on either male or female plants, but I have found, in one instance, antheridia and procarps on the same plant, which, however, did not mature any cystocarps.

I found that *Gr. confervoides* differed markedly in its minute structure from *Gr. multipartita* in having the intermediate layer much better developed. There is the same core of large cells, and the same superficial layer of small cells, but beneath the superficial layer is a layer at least three cells deep of more irregular and more loosely-packed cells, no doubt assimilatory in function (Fig. 15). There is also a less abrupt transition
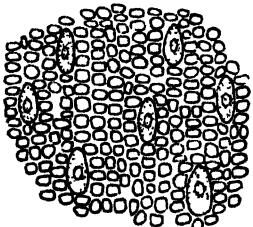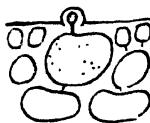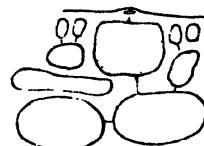
FIG. 11.   FIG. 12.   FIG. 13.   FIG. 14.

FIGS. 11-14. *Gr. confervoides.* Fig. 11. Showing surface view with carpogonia lying among the superficial cells. Fig. 12. Vertical section of carpogonium with restored papilla. Fig. 13. Vertical section (transverse) with indication of papilla. Fig. 14. Vertical section longitudinal) with similar indication.

from core-cells to intermediate cells than in *Gr. multipartita*. This difference of structure results in some difference in the early stages of the cystocarp as compared with *Gr. multipartita*.

No unicellular hairs arise in *Gr. confervoides* as far as I have been able to observe.

But in the occurrence of great numbers of carpogonia among the superficial cells of the female plants, both among the mature cystocarps and just behind the apex, the condition in *Gr. multipartita* is exactly repeated (Fig. 11). They present also the same signs of papillae on the middle point of the exposed surface; just behind the apex the papillae may be made out in profile view (Figs. 12, 13, 14), and often with granules of foreign matter attached. Here also I was able to show that these cells do not occur on tetrasporiferous plants, which I was not able to do in the other species.

Further, I was able with fresh material to verify the suspicion I had formed, from the study of *Gr. multipartita*, that the carpogonia, at the stage at which they are capable of fertilization at any rate, are devoid of the red colour of the neighbouring cells. As they are being differentiated behind the apex they gradually bleach, and if they are not fertilized, as the great

majority are not, they gradually reacquire some red colour in the derelict condition. It is noteworthy that both the male and female gametes are colourless in most Florideae.

I cannot altogether understand why these carpogonia have not been described by other observers. A transverse section of the female plant, drawn with the same detail as Thuret and Bornet (1878) have drawn the tetrasporiferous plant, must have revealed their presence, even though they were not taken to be procarps. In fresh material they are not so readily distinguished among the other superficial cells as they are in bleached material stained in Hoffmann's blue. It has occurred to me that they may have been seen and taken to be early stages of the tetrasporangia, with which they correspond precisely in situation, and this again may account for the statement referred to already, that tetraspores and cystocarps occur at times on the same plant, which at any rate I have never observed myself.

The process by which the fertilized carpogonium is overtopped by the rapid growth of the surface cells in its neighbourhood into vertical filaments is the same as that already described for the other species, and need not be described in detail. In the fact that there lies below the zygote a deeper layer of loosely compacted intermediate cells, above which the more compactly arrayed vertical threads arise, there is a difference. In *Gr. confervoides* the zygote lies farther from the large cells of the core, and at the boundary line between the older intermediate cells and the new cells of the proliferating surface (Fig. 15). The stimulation to growth causes hypertrophy among the contiguous cells of the intermediate layer, and the group of yellowish cells spoken of, by Thuret and Bornet as an early stage in the development of the cystocarp consists of cells from this layer, lying around and below the enlarged zygote, with which cells the zygote later on fuses. Before that stage is reached it has already taken on the same amoeboid appearance which I found in *Gr. multipartita*, and similarly preys upon the bases of the new threads lying above it. On this account a clear gelatinous interval soon appears immediately above the zygote, in which lie the remains of some of the disintegrating cells. This is the beginning of the appearance of a space which spreads by the solution of the bases of the new threads, forming what may be called a cavity (though filled with gelatinous substance), which is floored by intermediate cells, surrounding the developing zygote. Thus by the same two processes of solution of the cells above, and fusion with cells lying around and below, the zygote, or more properly fusion-cell, or better still trophocyte, reaches the stage when it is ready to bud off from its surface protuberances in which cell-walls appear, the new cells being the starting-points of the radiating and branching filaments of the carposporophyte (Figs. 15, 16, 17).

The cells of the intermediate layer which floor the gelatin cavity after

the dissolution of the bases of the new derivatives of the surface layer constitute what Johnson has labelled the placenta. This tissue takes no part directly in the production of carpospores, as Johnson supposed. Some of its cells, it is true, contribute to the nourishment of the zygote, many by undergoing absorption, several by incorporation bodily, but those that are left over are sterile, and ultimately all, or almost all, are sacrificed to the growing carposporophyte, which arises directly from the large trophocyte. The placenta, in the sense in which it is used by Thuret and Bornet, is the
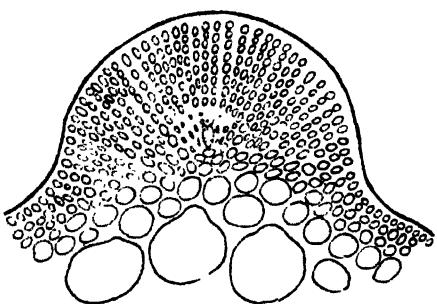
FIG. 15.   FIG. 16.   FIG. 17.

FIGS. 15 17. *Gr. confervoides.* Fig. 15. Vertical section through young cystocarp, showing situation of zygote and region of absorption. Fig. 16. Enlarged view of the same zygote, showing pseudopodial processes and the remains of absorbed cells of the vertical filaments. Fig. 17. Tangential section of zygote at the same stage.

vegetative body of the carposporophyte itself, derived solely from the fusion cell.

Johnson finds a spore cavity which arises in the gelatinous space above the growing zygote. I find no such cavity in my preparations, and am of opinion that it is due in his material to shrinkage of the diffluent gelatinous substance in spirit. It is difficult to conceive of schizogeny, such as he bargains for, occurring in such a tissue. The tissues of the mature carposporophyte in *Gr. confervoides* bear the same relation to those of the gametophyte as they do in the case of *Gr. multipartita.* The placenta is less compact, and the intermediate cells below the spore-chains are much more voluminous than in *Gr. multipartita.*

It will be remembered that, in the account given by Johnson (1887) of the evolution of the cystocarp of *Gr. confervoides*, there arises *after* the appearance of a group of procarpial cells, and *after* the solution of cells above and about them, whereby a clear gelatinous space is produced, a carpogonium which is extended into a trichogyne. The identification of a two- or three-celled carpogonial branch, and of certain auxiliary cells, is somewhat hazardous, judging from the figure. But it would seem difficult to gainsay the recognition of a trichogyne, which is shown in more than one

figure. Naturally, if my identification of the carpogonium, as occurring among the superficial cells, where the thallus is still unswollen, is correct, there ought not to be a trichogyne produced at the stage figured by Johnson, for fertilization will previously have been effected. I may say, however, that I looked diligently for a trichogyne at this later stage, on the supposition that the papilla might have lengthened *pari passu* with the proliferating surface, but failed to find any trace of it. How then are the appearances figured by Johnson to be accounted for? He himself has mentioned one possibility of error, but considered that he had guarded himself against misinterpretation on this score: ' Threads crossing the fruit-cavity before any spores are often seen. . . . It is necessary to exercise care in order to avoid confounding these with the trichogyne.' There is another possibility of error (or possibly it is the same) mentioned by Thuret and Bornet (1878): ' There are sometimes observed filaments which extend like columns between the placenta and the pericarp. They are hyaline, elongated cells analogous to those adventitious, descending hairs so frequent among the algae, and emanate either from the placenta or from the vault of the pericarp.' I have already referred to finding in *Gr. multipartita* strong threads arising from the carposporophyte which cross over to the canopy and prey upon its cells. I considered them as an additional means of obtaining nutriment for the sporophyte, resorted to in that species in the later stages of the development of the cystocarp. I believe these columns in *Gr. confervoides* to be of the same nature (Fig. 18). If so, they are ascending, not descending, filaments, for they arise from the placenta of the sporophyte. I have not seen them, it is true, at this early stage, where the trichogyne is figured by Johnson, but very little later they occur in considerable numbers, especially on the flanks of the carposporophyte. Now if such an ascending thread occurred just at the highest point of the developing sporophyte, it might easily be taken to be a trichogyne. Failing these explanations of the appearances figured, I am at a loss to account for them.

I might urge a more general consideration against the trichogyne-like structure being regarded as a true trichogyne. In all the cases known to me, the trichogyne arises at the surface, and if it is later submerged, it is the result of growth round it. In this case, however, the trichogyne would have to perforate its way, like a fungal hypha, through a fairly compact tissue ten to twelve cells deep. Though such a thing is conceivable, it is at any rate unlikely, and certainly without precedent in the red algae. The protruding papilla *t'* represented in Johnson's Fig. 2 I take to be the papilla of the early carpogonium still showing on the raised cuticle, or perhaps the remains of a spermatium (Fig. 5).

To sum up now the results of my observations on *Gr. confervoides*. I find the course of events is essentially the same as in *Gr. multipartita*.

The procarp consists of a carpogonium surmounted by a papilla. As the result of a stimulation presumably following fertilization, the carpogonium, at first superficial, is embedded in a tissue several cells deep. Here it begins to prey upon the cells surrounding it, fusing with some, and dissolving and absorbing the contents of many more by sending pseudopodial processes among them to a considerable distance. As a result it becomes a great fusion mass lying in a space which it has excavated for itself at the base of the vertical cells. It now buds off from all parts of the surface, excepting the base, where it is still attached to a stalk-cell, groups of cells which are the apical cells of the more or less spherical carposporophyte.

FIG. 18. *Gr. confervoides.* Vertical section of developing cystocarp, showing carposporophyte with superficial spore-chains, and carposporophytic threads growing into the canopy for purposes of nutrition.

A good figure of a young stage in the growth of the carposporophyte is given by Thuret and Bornet (1878), Pl. XL. Fig. 5.

I am aware that there are still left considerable gaps in our knowledge of the reproduction of *Gr. confervoides.* The adhesion of the spermatium to the papilla has to be confirmed, the fusion of the sexual nuclei to be observed, and in particular the cytology of the zygote and carpospore requires investigation, and that of the tetraspore no less. I have found that microtome sections obtained by freezing soft material afford the best material for the study of the minute morphology of the tissues, but they do not suffice for cytological observations. On the other hand, sections obtained from dehydrated material cut in paraffin are the only means for the adequate study of the nucleus, but are difficult of interpretation from the point of view of minute morphology. The two methods seem to me to be complementary. I am glad to know that my friend Mr. W. Matthias, B.Sc., contemplates the study of the cytology of *Gr. confervoides.* The identification of the carpogonium is at any rate a necessary starting-point for any such study.

3. *Gracilaria compressa*, Grev.

Although I have thought that some of the forms of *Gr. confervoides* found on the coast of Anglesey approach *Gr. compressa* very closely, I have

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also examined herbarium material of typical *Gr. compressa* from the south of England, and have satisfied myself that papillated carpogonia occur in great numbers on the female plant in this species also. In the dried material, mounted after warming in dilute glycerine, the carpogonia were found to be devoid of red chromatophores, and the papillae were even more readily demonstrable than in material preserved in spirit.

I do not propose to enter here upon a discussion of the systematic position of *Gracilaria* as affected by the results here disclosed. It is clear that the inclusion of the genus in the same family with *Sphaerococcus* (Schmitz and Hauptfleisch (1897)), with a central thread type of structure and a four-celled carpogonial branch, and *Calliblepharis* (Phillips (1897)), with a fountain type of structure and a three-celled carpogonial branch, cannot be longer justified. More than that, *Gracilaria* would seem to present in the structure of its procarp a great contrast to that of all other Florideae, and an approximation to that of the Bangiaceae, notwithstanding the fact that the subsequent development of the zygote is very different. Schmitz (1893) laid great stress upon the absence of a carpogonial branch and a true trichogyne in Bangiaceae. It would seem that they are equally absent in *Gracilaria*, which has nevertheless all the other marks of the Florideae. The question of the clean separation of the Bangiaceae from the rest of the Rhodophyceae would thus seem to be reopened.

SUMMARY.

1. The procarps in *Gracilaria multipartita*, *Gr. confervoides*, and *Gr. compressa* are dispersed in great numbers over most of the surface of the adult female plants, and do not arise first within the cystocarpial swelling.

2. The procarps consist simply of a carpogonium with an apical papilla. There is no carpogonial branch, and no elongated trichogyne.

3. In *Gr. multipartita* and *Gr. confervoides* fertilization is followed by the formation of the cystocarpial swelling, and the zygote is embedded at the base of the proliferated tissue.

4. Here it enters upon a course of parasitism, fusing with a great number of cells, and bringing about the solution of many more, the contents of which it absorbs. There are no true auxiliary cells.

5. After adequate nutrition, it buds off cells from its surface, growing into the space it has excavated, and ultimately giving rise to carpospores at the surface of the carposporophyte within a dome-shaped cavity.

6. The stomium is formed by solution and internal pressure at the top of the dome.

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A Side-issue of the Age-and-Area Hypothesis.

BY

H. B. GUPPY, M.B.

D R. WILLIS in developing the argument of his theory laid stress on
the support given to his views by the singular relation between a
mainland flora and the floras of off-lying islands, the species common to the
mainland and the islands having wider ranges on the mainland than the non-
insular species. This relation between range in a mainland and distribution
outside presented itself so frequently that an explanation had to be found,
and he found it in his ' Age-and-Area ' hypothesis. Thus in the case of
Ceylon he found that whilst the endemic species in that island occupied on
the average the smallest areas, the species that extended to peninsular India
had rather larger ranges in Ceylon, and those ranging beyond the peninsula
occupied the largest areas of all. The same principle was presented by the
floras of the main islands of New Zealand and the floras of the off-lying
islands, the Chathams, the Aucklands, the Kermadecs, &c. Here the species
of the islands ranged on the average nearly 300 miles more in New Zealand
than the species which did not reach the islands. Exactly parallel results
were obtained in the case of the floras of the British mainland and the floras
of the various islands off the coasts, from the Orkneys to the Scillies. In
this last case it was brought out that if we take at random the families
Ranunculaceae, Caryophyllaceae, Leguminosae, Orchidaceae. and Grami-
neae, the average distribution of a species in Great Britain is 47 out of
Watson's 112 vice-counties, but for the species occurring on the islands the
average distribution in Great Britain is 71 vice-counties.

Working on the basis of his theory Dr. Willis was able to predict most,
if not all, of the general results obtained. For example, it was predicted and
verified that the outlying islands of New Zealand would have a flora that
was very old in New Zealand and therefore very widespread there. The
same thing was done and the same inference drawn for the floras of the
islands off the British coasts. Both in the case of New Zealand and in that
of Great Britain it was shown by him that wide dispersal among the islands

meant wide range and great age on the mainlands, the age determining the range, as implied in his theory.

This relation between range within the island and range outside it might be differently explained if it was concerned with the colonization and occupation of a new territory by a dominant race of men as in the case of the Norman invasion of Britain. Here area goes with dominant racial qualities; and may it not be the same with migrating plants? The distribution in Britain of the hardy species of *Ranunculus* may be due as much or more to generic qualities, and these qualities might explain the behaviour of the genus in New Zealand. If a species was hardy enough to establish itself on the Chatham Islands and the Macquarie Islands off New Zealand, the same qualities would explain its wide range in the main islands. Any correlation of age with range may, as Thoday points out in his recent paper on *Passerina* in South Africa (this Journal,' vol. xxxix, January, 1925, p. 199), be greatly complicated by the operation of other factors. Thus it may be cancelled out by Age itself according to the principle suggested by Wallace in his ' Island Life ' (6), where Time ' discounts the means of dispersal ' and the Tortoise draws up with the Hare in the race for Area. But these other factors are allowed for by Willis himself, as in the case of *Eugenia* in Ceylon, where adaptivity is regarded as a necessary quality for the extension of the range of the species (7, p. 59). ' Age ', he adds (7, p. 61), ' can in itself effect nothing, but it allows time for the various factors that are active in distribution to produce their effects.' This opens a wide gate for the intrusion of various subsidiary principles into the problem of plant-distribution when regarded as determined by Age, and one of the foremost of these would be the qualities of hardiness and adaptivity acquired by plants that have survived ages of migrations. Such plants largely compose the British flora, and the qualities acquired by them during the age-long vicissitudes of climate and locality have made them the possessors of our land as surely as similar qualities ensured the success of the various races of men invading Britain.

This point of view was developed while the writer was endeavouring to apply the ' Age-and-Area ' theory to the Cornish flora. He found that the plants of the Scilly Islands present the same relation to the flora of the Cornish mainland that the flora of Ceylon presents to that of the Indian peninsula, and the floras of the outlying islands present to those of the main islands of New Zealand. But he went a step farther back, and on inquiring into the characters of the Scillian flora he found in those islands a gathering of plants that in many cases had long since proved their capacities for extending their ranges by travelling over much of the globe.

The relation between the Scillian flora and that of the Cornish peninsula is this. Having gone through Davey's admirable ' Flora of Cornwall ' (1), a work supplying an abundance of data for determining this point, the

writer discovered that the large number of Scillian plants existing on the Cornish main had a much greater average range in the county than the Cornish plants not recorded from the Scilly Islands. For this purpose Davey's eight districts of the county were employed, and whilst the non-Scillian plants had an average range in the Cornish main of 5·2 districts, that for the Scillian plants in the peninsula was as much as 7·3 districts, or nearly half as much again. This contrast displayed itself consistently throughout the systematic scale from the Ranunculaceae to the Ferns.

Here the writer's investigation would have ended had it not been for the unwillingness on the part of some Cornish botanists to accept such a relation of the Scillian flora as evidence on behalf of a theory which for them at any rate could never be proved. This led to his looking around for another road of approach to the problem, and this again led to the discovery of an alternative view of the significance of this relation, the nature of which has already been indicated. A recent perusal of the two papers of J. R. Matthews on the Perthshire flora from the point of view of the hypothesis of Willis had suggested one or two other means of comparison, and one of them was to apply the results of the Census of Comital Distribution given in Watson's ' Topographical Botany ' to the flora of the Scilly Islands. The results of this census are given in a convenient form in Dr. Druce's ' List of British Plants '.

After preparing a list of British plants that occur in all the 112 vice-counties of Great Britain, the writer found that the 124 species concerned fell readily into two principal groups : the Agrestal and the Paludal. The term Agrestal, which he has borrowed from Dr. Druce, is used in its widest sense to cover all plants growing habitually on ground in agricultural use or which ' owe their persistence to man and his operations ', and accordingly it includes the large Viatical Group of Dr. Druce. The Agrestal Group thus extended holds 68 per cent. of the total, a proportion that would be nearer 80 per cent. if we include the Paludal plants that have been affected by man's agricultural operations. The Paludal Group holds about 23 per cent. of the total of plants occurring in all the 112 British vice-counties of Watson. They are probably the oldest and the most truly British plants of our flora and include all the plants growing in moist stations. The designation is borrowed from Dr. Druce's list. The natural division of British plants into two chief groups, the Agrestal and Paludal, epitomizes most of the history of the flora. The distribution of the Scillian and non-Scillian plants in the Cornish peninsula reflects the distribution of the same plants over the length and breadth of Britain. Just as these two groups of plants have an average distribution in Cornwall of 7·3 and 5·2 districts respectively, so if we take as our guide Watson's census of the British flora we find that their average comital distribution in Britain is 84 vice-counties in the case of the Scillian plants and 56 for the non-Scillian plants, the two

sets of values displaying a parallelism which is highly significant when we come to look for a clue to the greater ranges of Scillian plants in the Cornish main.

Like the 124 plants of maximum distribution in Britain (those occurring in all the 112 vice-counties), the Scillian plants of the Cornish peninsula are largely Agrestal, one-fourth of them being maximum-range plants, whilst the non-Scillian plants show only 13 per cent. of these plants. The Agrestal character of the Scillian plants in the Cornish main is emphasized in a rather curious fashion by the predominance of Azorean plants in the Scillies as compared with Cornwall proper. It is a remarkable fact that the flora of the Scilly Islands is much more Azorean than that of the Cornish peninsula, almost half (49 per cent.) of the Scillies being Azorean species, whilst only one-seventh of the purely Cornish plants are held in common with the Azores. (The Azorean flora, as indicated below, is mainly Agrestal.)

SUMMARY.

The flora of the Scilly Islands presents the same relation to the flora of the Cornish mainland that the floras of the outlying islands of New Zealand present to the floras of the main islands, and the flora of Ceylon presents to that of the Indian peninsula. This relation of the Scillian flora to the flora of the mainland of Britain was specially indicated by Willis in his work on the 'Age-and-Area' theory (7, p. 70), where he showed that plants of the Orkneys, Colonsay, Clare Island, and the Scillies had an average of 50 per cent. greater range in Britain than the average range of British plants. Dr. Willis chose at random the families Ranunculaceae, Caryophyllaceae, Leguminosae, Orchidaceae, and Gramineae, and he explained the greater range of the insular plants in terms of his theory, range going with age within a district. This principle runs consistently through the Scillian flora, the areas of Scillian plants on the Cornish mainland being on the average half as great again as those of the non-Scillian plants. On finding that the Scilly Islands hold an unusual number of plants widely distributed in Britain, the writer considered that the wider range of Scillian plants in Cornwall might be thus accounted for, the plants concerned behaving in Cornwall exactly as they do in Britain. They are mainly Agrestal, that is, plants growing habitually on ground used in agriculture or in soil disturbed by man.

This explanation would imply that the greater ranges of insular plants on a mainland depends on their adaptivity, hardiness, and fitness for dispersal, rather than on age within the region. The plants that are widest distributed in Britain are those that have acquired special fitness for dispersal during ages of migration, qualities that have carried them over the world.

It is these plants that have largely displaced the native plants of the Azores, and hence it is that the Scilly Island flora is more Azorean in character than the flora of the Cornish peninsula.

The writer suggests that the mysterious flora of the Californian coast islands might be found to respond like that of the Scilly Islands to the same treatment.

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Chemical Studies in the Physiology of Apples.

IV. Investigations on the Pectic Constituents of Apples.

BY

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With two Figures in the Text.

INTRODUCTION AND HISTORICAL RÉSUMÉ.

THE class of substances known as the pectins or pectic compounds have been vaguely recognized, and as vaguely studied, by a number of botanists and chemists for over a hundred years. They are described as colloidal gelatinous substances of carbohydrate nature, occurring in varying amounts in herbaceous trees and plants, in the tissues of fruits. in certain roots such as carrots and turnips, and as constituents of gums and mucilages.

During recent years the significance of the pectic compounds in relation to normal and abnormal processes of plant life has been considered, and their importance in connexion with certain economic problems has also been recognized. Several lines of investigation have received attention, and the following may be enumerated as the most important :

(1) The relation of pectic metamorphosis to fungal and physiological disease.

(2) The significance of the pectin content of fruits in the setting of jams and jellies.

(3) Problems connected with fermentation processes in the manufacture of wine, beer, and cider.

(4) The importance of the reactions and decomposition products of pectic substances in the preparation of fibres used in textiles.

(5) The relation of pectic compounds of fruit to the metabolic changes which it undergoes during ripening and death. From this arises subsidiary problems of commercial importance, as, for instance, the connexion between the pectic content of the fruit and its keeping properties in the various types of storage.

Hence the necessity has arisen for detailed knowledge of the nature and constitution of the pectic compounds, and for the development of accurate methods for their extraction and estimation from plant and fruit tissues.

The literature on the subject is confusing and contradictory, and, in view of the renewed interest, it will be useful to give a brief account of the main conclusions arrived at by earlier investigators.

The Nature and Distribution of the Pectic Compounds.

In 1833 Braconnot (6) discovered a gelatinous substance in vegetable tissues, which he called pectic acid, and his discovery was followed up by Vaquelin and Mülder, who investigated the properties of this acid, and also described the presence of a soluble related substance in various fruit juices to which they give the name of pectin.

Later, Payen (1856) (26) isolated pectic acid in combination with calcium, and suggests that a calcium pectate forms the basis of the middle lamella of plant tissues.

In 1848 Frémy (18) published memoirs describing a whole series of eight related pectin compounds. He examines the properties of these compounds, and attempts to elucidate their constitution and relationship to one another.[1] Frémy also describes a parent substance, ' pectose ', associated with cellulose in the cell-walls in an insoluble condition, and which he regards as a calcium salt. He considers that, during the ripening of fruits, pectose is progressively decomposed into soluble pectin by the acid in the cell sap. According to Bourquelot and Hérissey (1898–9) (4), however, the conversion of pectose into pectin is a result of enzyme activity.

Frémy suggests that, as ripening proceeds, pectin itself is decomposed into a series of pectic acids by the action of enzymes. These break-down products vary in properties and composition, and finally result in the stable end-product of the series, which he calls metapectic acid.[2] Frémy points out that a similar decomposition can be artificially brought about by prolonged treatment of the fruit with dilute organic acids. The pectin in the resulting extracts can be precipitated by the addition of alcohol. A considerable amount of calcium is also brought into solution by this method, and Frémy therefore concludes that pectose is a calcium compound of pectin. This view was opposed by Wiesner (1861) (37), who suggested that

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[1] In view of the more recent work, it is highly improbable that these numerous pectins described by Frémy have actually a separate existence.

[2] See foot-note on next page.

pectose was a compound of pectin with cellulose, and this view has been upheld by later investigations.

As a result of these preliminary researches, various workers attempted to elucidate the nature and distribution of the pectic compounds of plant tissues. The majority of investigators locate the pectic substance in the cell-wall, and especially the middle lamella, but the development of the problem was greatly hampered by the then prevailing conception of the uniform nature of the cell-wall.

The next development of any importance is due to Mangin, who published (1891–3) (23) a detailed account of his investigations on the nature and distribution of the pectic compounds in various plant tissues. Following the nomenclature suggested by Frémy, Mangin divides the pectic compounds into two series—those of neutral reaction, notably pectose and pectin, and those exhibiting acidic properties which include the various pectic acids described by previous investigators. Mangin found that the members of both series are closely related, since, by mild treatment of the tissues with acid or alkali, the pectose which he regards as a cellulose compound can be transformed into pectin, pectin into various pectic acids, the ultimate product of pectin decomposition, using excess of alkali, being a stable one which Mangin considers to be the soluble ' metapectic acid ' described by Frémy. Mangin isolated this acid and obtained from it, on further decomposition with sulphuric acid, a sugar which he thought to be arabinose, and a complex organic acid which he was unable to identify.[1] Mangin therefore considers that pectose, pectin, pectic acid, and the stable end-product, metapectic acid, are the only members of the series which are definite compounds exhibiting constant properties and composition. The numerous modifications described by Frémy he regards as unstable intermediate products arising from the progressive decomposition of the more stable forms.

Mangin agrees with the hypothesis put forward by Frémy, that the production of pectin is an important factor in the ripening of fruit, and considers that the development of the fruit is associated with fundamental changes in the pectic constituents of the tissues. Mangin makes some attempt to locate the different forms of the pectic bodies in various plants, basing his investigations on a combination of two methods :

(1) The use of stains such as safranin, methylene blue, and, in his later work, ruthenium red, in order to differentiate the pectic compounds from the other constituents of the cell-walls.

(2) The application of chemical reagents to confirm the results obtained by staining methods only.

[1] It is difficult to decide, from the data supplied by Frémy and Mangin, the real nature of this ' metapectic acid ', and whether they are identical products. In view of the work carried out by Ehrlich and Fellenberg (pp. 815–819), it seems probable that metapectic acid corresponds either to galactose, galacturonic acid, or to *d*-tetra-galacturonic acid.

The principle underlying the chemical method depends upon either the solution of the cellulose by Schweitzer's reagent, in which the pectic compounds are insoluble, or upon the converse method of removing the pectic substances by reagents known to effect their solution, the structure of the residual tissue being maintained by cellulose only. By a combination of both these methods, Mangin was able to observe the disposition of whichever constituent had been left undissolved, and consequently to form a conception of the relative distribution of the cellulose and pectic material in the tissue structures. He concludes from his experimental evidence that pectose is intimately associated with cellulose in the substance of the cell-walls, and cannot be separated from it in an unchanged condition. He finds that the lining of the intercellular spaces and the middle lamellae are composed of pectic material, and agrees with Payen that the latter are constituted of a calcium salt of pectic acid.

Mangin's researches consequently contributed valuable evidence to the theory of the heterogeneity of the cell-wall.

The Chemical Constitution of the Pectic Compounds.

The chemical composition and constitution of the group of pectic substances had meanwhile attracted very little attention, probably owing to the difficulty of isolating the individual pectic compounds from the tissues in a sufficiently pure condition for analysis. The following account enumerates the more important results obtained by the early investigators.

Some light was thrown on the constitution of the pectic compounds by Herzfeld (1890-1) (20), who showed that they contained both pentose and galactose residues. Scheibler (1868) (27) found that an acid and reducing sugars resulted from hydrolysis of Frémy's ' metapectic acid ', and Mangin obtained similar results.

The observations of Scheibler, Herzfeld, and Mangin were confirmed by Bauer (1894) (3), who identified both xylose and arabinose as cleavage products of pectin and pectic acid, and later Tromp de Haas showed that hexoses, in addition to pentoses, were present.

Tromp de Haas and Tollens (1895) (32) attempted numerous analyses of pectin from various sources, and obtained results approximating to the atomic ratio CH_2O, and therefore concluded that pectin is related to the carbohydrates. Tollens (31) observed later that the percentage of oxygen is invariably slightly higher than is found in the case of the carbohydrates, and accounts for the difference by supposing a carboxal group to be present in pectin. He suggests that the carboxal occurs in the pectin molecule as an acid of the nature of glucosonic acid, neutrality of the pectin being accounted for by supposing lactone formation to occur. Tollens (1901) also puts forward the alternative suggestion that the acid combines with the —CHO or CH_2OH group of the sugars to form esters. In both cases hexoses,

pentoses, and free acid would obviously result from hydrolytic treatment of the pectin. Tollens suggests for this acid, which he calls pectic acid, the formula $C_{17}H_{24}O_{16}$.

The constitution of the pectic substances attracted no further attention till 1916, when a systematic chemical research was published by Schryver and Haynes (1916) (28).[1] These authors obtained soluble pectin from various sources, and found that treatment with caustic alkalis converted all the samples into a pectic acid of uniform composition. Analysis of the pectic acid thus obtained corresponded 'very closely with a definite chemical formula containing seventeen carbon atoms (or a multiple thereof)', and Schryver and Haynes therefore give pectic acid the empirical formula $C_{17}H_{24}O_{16}$.

Subsequent analyses of pectin showed that its composition was approximately the same as that of pectic acid, and therefore its empirical formula also corresponds to $C_{17}H_{24}O_{16}$.[1] Schryver and Haynes detected pentose groups in both pectin and pectic acid by estimating the furfural obtained by distillation of pectin with hydrochloric acid (Tollens' phloroglucinol method).

Further researches on the constitution of the pectic compounds were carried out by Ehrlich (1917) (14) in the course of an investigation of their possible food value.

By hydrolysis of the water-insoluble residue of beets, Ehrlich obtained a substance which he found to consist of arabinose and a compound of pectic nature apparently combined with methoxy groups and with calcium and magnesium. He therefore concludes that this substance is a neutral CaMg salt of pectic acid, containing methoxy groups combined as esters and associated with arabinose.[2]

Ehrlich submitted this compound to hydrolysis, comparing the effect of oxalic acid and caustic soda.

Oxalic acid split off the calcium, magnesium, and ultimately the methoxy groups, and an acid remained which corresponded to the formula $C_{12}H_{20}O_{12}$ and yielded a large amount of mucic acid on oxidation. From this and other considerations, Ehrlich considers it to be a galactose-galacturonic acid, formed by the combination of both these substances, with elimination of water, in the following manner :

$$C_6H_{10}O_7 + C_6H_{12}O_6 = \underbrace{C_6H_9O_7 - C_6H_{11}O_5}_{C_{12}H_{20}O_{12}} + H_2O$$
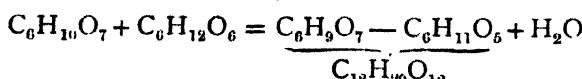
Treatment with soda had a more violent hydrolytic effect, the galactose

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[1] The pectinogen and pectin described by Schryver and Haynes correspond to pectin and pectic acid respectively, according to the nomenclature adopted in this communication.

[2] The nomenclature adopted by Ehrlich is confusing, but examination of his experimental details suggests that this so-called neutral salt of pectic acid corresponds with soluble pectin.

being split off as well as the calcium, magnesium, and methoxy groups, leaving free galacturonic acid. The resulting product has much stronger acidic properties than the galactose-galacturonic acid, and corresponded to the formula $C_{24}H_{34}O_{25}$. Ehrlich calls this compound 'tetra-galacturonic acid' and considers it to originate from four molecules of galacturonic acid, with elimination of three molecules of water.

$$4C_6H_{10}O_7 - 3H_2O = C_{24}H_{34}O_{25}.$$

The results obtained by Ehrlich may be summarized as follows:

Hydrolysis of the Pectic Substances in Beet.

Pectin (arabinose + CaMg salt of the methyl ester of galactose-galacturone and acid).

Oxalic |
  acid. ↓
    Galactose-galacturonic
      acid. $C_{12}H_{20}O_{12}$
Caustic |
  soda. ↓
    Tetra-galacturonic
      acid. $C_{24}H_{34}O_{25}$
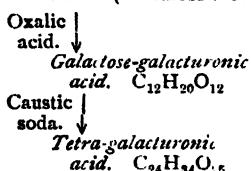
In 1918 Fellenberg (17) published a detailed account of the chemical properties and constitution of the three pectic compounds which he regards as having an independent existence, namely—pectose, which he alludes to as 'protopectin' (in accordance with the earlier work of Tschirch (1908) (33)), pectin, and pectic acid.

Protopectin or Pectose.

In accordance with the work of earlier investigators, Fellenberg (17) regards 'protopectin' as the insoluble precursor of the soluble pectin, and suggests that it is either a simple anhydride of pectin or a complex compound consisting of pectin in combination with cellulose in accordance with the opinion of earlier investigators, Mangin (23), Wiesner (37), Haas and Tollens (31). His investigations demonstrate the presence of methoxy groups in pectose (protopectin), but, as it could not be extracted unchanged, Fellenberg was unable to establish any definite relationship between the methoxy content and the amount of pectose present in the tissues.

Pectin.

In order to obtain more accurate knowledge of the constitution of pectin, Fellenberg adopted a method originated by Bourquelot and Hérissey, for the preparation of pure samples of pectin.

After preliminary extraction of the tissue with alcohol the residue was treated with superheated steam, and the resulting pectin precipitated with alcohol. Fellenberg showed from analysis of pectin that, in addition to the

arabinose, galactose, galacturonic acid, and methoxy groups detected by Ehrlich and previous investigators, methyl pentose was an invariable constituent of the pectin molecule. On the other hand, Fellenberg could not obtain confirmation of Ehrlich's conclusion that pectin was combined with calcium and magnesium, and considers them to be present as impurities.

Ehrlich had already shown that the methyl groups in pectin could be removed by hydrolysis with alkali, and less readily by oxalic acid (pp. 815, 816). Fellenberg confirmed these results, and showed that the methyl groups were split off as methyl alcohol, the amount produced from the pectin depending upon the strength of the hydrolytic agent, alkalis being more powerful in this respect than acids.[1] Fellenberg therefore suggests that it should be possible to remove the methoxy groups progressively, and so obtain pectins of lower methoxy content as intermediate products between neutral pectin and pectin acid.

As a result of numerous analyses, Fellenberg deduces that pectin arises from the coupling up, with elimination of water, of two molecules of arabinose, one molecule of methyl pentose, one molecule of galactose, and eight of galacturonic acid in the form of its methyl ester. He therefore suggests the following provisional formula for pectin, representing the neutral octomethoxy form :

$$(C_5H_8O_4)_2(C_6H_{10}O_5)_2(C_5H_7O_4—COOCH_3)_8 . 2 H_2O,$$

or the empirical formula, $C_{73}H_{120}O_{68}$.

From this neutral form a series of pectins with decreasing methoxy content and increasing acidity was obtained by hydrolysis, the completely demethoxylated pectin being pectic acid with eight carboxyl groups. Fellenberg suggests that these products intermediate between pectin and pectic acid should be called *pectinic acids*.

In support of his formula, Fellenberg obtained pectins from different sources of which the varying methoxy content determined experimentally corresponded approximately to those calculated from the theoretical formula

$$C_{62}H_{96}O_{52}(COOCH_3)_n(COOH)_{8-n}.$$

Analyses of the elements also agreed satisfactorily with the theoretical formula for pectin, $C_{73}H_{120}O_{68}$.

Fellenberg therefore concludes that the various pectic compounds described by Frémy, Mangin, and other earlier investigators may be regarded as pectin derivatives of varying methoxy content, which accounts for the numerous products obtained with only slightly varying properties.

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[1] Methyl alcohol as a product of the hydrolysis of pectic substances has been detected by other investigators: Wolf (1900) (40), Lippmann (1920) (22), Tutin (1921) (34), Hornby (1920) (21), Sucharipa (1924) (29).

Pectic Acid.

Fellenberg therefore deduces from the above considerations the following formula for pectic acid, $C_{62}H_{96}O_{52}(COOH)_8$, derived from pectin by complete hydrolysis of the methoxy groups :

$$C_{62}H_{96}O_{52}(COOCH_3)_8 + 8\ H_2O = C_{62}H_{96}O_{52}(COOH)_8 + 8\ CH_3OH.$$
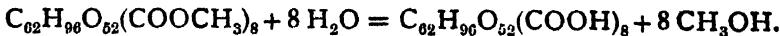
The accuracy of this formula was tested by showing that the conversion of pectin into pectic acid was a quantitative reaction. Fellenberg suggests that confirmation could also be afforded by analyses of the various salts of pectic acid. For example, the barium salt $C_{62}H_{96}O_{52}\left(\genfrac{}{}{0pt}{}{COO}{COO}{>}Ba\right)_4$ has a theoretical barium content of 21·36 per cent., and Fellenberg obtained 22·3 per cent. by experimental estimation, which he considers to agree with the calculated result.

On the other hand, the 22·3 per cent. barium obtained by Fellenberg's experiment agrees more closely with the 22·13 per cent. demanded by the formula for barium pectate suggested by Schryver and Haynes, i.e. $C_{68}H_{88}O_{64}Ba_4$. Similarly, calcium pectate according to Fellenberg's formula has a theoretical calcium content of 7·33 per cent., whereas the mean of 30–40 analyses of the compound from many different sources and Haynes was 7·64 per cent. (8), which agrees very closely with the 7·57 per cent. required by the Schryver and Haynes formula, $C_{68}H_{88}O_{64}Ca_4$.

It appears conclusively that the formula for pectic acid deduced by Schryver and Haynes has more support from experimental evidence than that put forward by Fellenberg.

It is suggested, however, that the Schryver and Haynes formula for pectic acid, i. e. $C_{17}H_{24}O_{16}$, 'or a multiple thereof, is actually comparable to that of Fellenberg—since the multiple $4(C_{17}H_{24}O_{16})$ or $C_{68}H_{96}O_{64}$ approximates to the Fellenberg formula $C_{70}H_{104}O_{68}$.

The difference in the carbon numbers is within the limit of experimental error, and the lower hydrogen and oxygen content of the Schryver and Haynes multiple formula can be accounted for by supposing the Fellenberg formula to contain four additional molecules of water. In other words, the Fellenberg formula may be regarded as the normal acid with eight carboxal groups, and the Schryver and Haynes formula as the anhydride of the acid formed by the elimination of four molecules of water from the eight carboxal groups

$$[4(C_{17}H_{24}O_{16})]\ \text{or}\ \ C_{68}H_{96}O_{64} = [C_{70}H_{104}O_{68}-4\ H_2O]\ \text{or}\ \ C_{70}H_{96}O_{64}$$

  (Schryver and Haynes)   (Fellenberg).
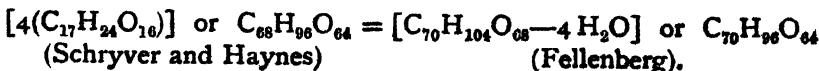
The results obtained by Ehrlich are not incompatible with those of Fellenberg, as at first appears. Fellenberg converts pectin into a pectic acid which is merely a demethoxylated form of pectin, but the products

of hydrolysis obtained by Ehrlich are forms of pectic acid which appear to be related to the Fellenberg product, but from which not only the methyl groups but also the other constituent groupings are removed.   It is possible that Ehrlich used a more vigorous process of hydrolysis than that employed by Fellenberg, which resulted in this further decomposition of the pectic acid molecule.

The following formulae represent the succession of products obtained by Fellenberg and Ehrlich from the hydrolysis of pectin as their results are interpreted by the present writer :

Hydrolysis products of pectin obtained by :—

Fellenberg
- *Pectin* $(C_5H_8O_4)_2(C_6H_{10}O_5)_2(C_7H_7O_4—COOCH_3)_n . 2H_2O$
- *Pectinic acids* $(C_5H_8O_4)_2 C_6H_{10}O_5)_2(C_5H_7O_4[COOCH_3]_n[COOH]_{8-n})$
- *Pectic acid* $(C_5H_8O_4)_2 C_6H_{10}O_5)_2(C_5H_7O_4—COOH)_8 . 2H_2O$

Ehrlich
- *Galactose-galacturonic acid* [1] $C_6H_{11}O_5 . OOC . C_5H_9O_5$ or $C_{12}H_{20}O_{12}$
- *Tetra-galacturonic acid* [1] $4 C_5H_9O_5 . COOH . 3H_2O)$ or $C_{24}H_{34}O_{25}$
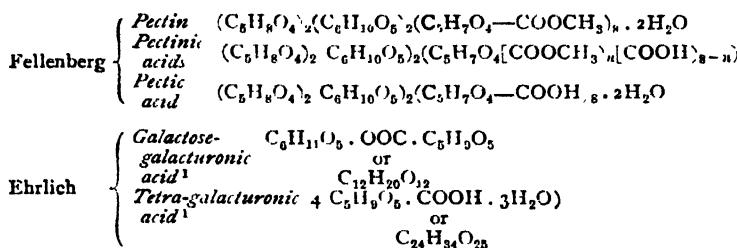
More recent work carried out by Sucharipa (1924) (29) has been directed to an investigation of the nature of pectose (protopectin) obtained from lemons.   He demonstrates that progressive hydrolysis of pectose results in the simultaneous production of pectins and free cellulose, and deduces from his experiments that pectose is not a homogeneous substance, but contains a series of pectins in which the methoxyl groups are more or less completely replaced by the cellulose radical.

The pectin developed in the ripening fruit Sucharipa calls ' free pectin ', and finds that it can easily be removed from the tissues with cold water. Estimation of the methoxy content of this ' free pectin ' shows it to contain the highest percentage of methyl alcohol, i.e. 11·33 per cent.   This figure agrees well with the 11·62 per cent. methyl alcohol obtained by Fellenberg for neutral octomethoxy pectin.   Sucharipa then separated the residual pectose (or protopectin) by dissolving out the cellulose with Schweitzer's reagent, and decomposed the product by various methods, heating with water under pressure, boiling with 50 per cent. sucrose solution (a method recommended by Tschirch), and heating with ammonium oxalate solution. The products of hydrolysis of pectose thus obtained yield fractions with varying methyl alcohol content according to the method of procedure, but the last and most stable fraction, extracted only by means of boiling with ammonium oxalate, was poorest in yield of methoxy groups and presumably

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[1] The metapectic acid described earlier by Frémy and Mangin apparently corresponds to one of these two compounds ; see pp. 812 and 813.

owing its stability to the number of cellulose groups with which the pectin residue was combined. The yield of methyl alcohol obtained from these fractions agrees closely with the values obtained by Fellenberg for the various pectinic acids derived from the neutral octomethoxy pectin (see p. 817).

Pectose therefore cannot be regarded as a substance of invariable composition, but as a compound of methoxylated pectin where any number from one to eight methoxy groups may be replaced by cellulose groupings. The final product to be extracted by Sucharipa, however, corresponds to a pectose containing *one* methyl group and *seven* cellulose residues. Hence, methods of artificial hydrolysis of pectose will produce products of varying composition, i. e. neutral pectin itself or any of the pectinic acids, according to the power of the agent employed.

The *natural* decomposition of pectose which is observed in ripening fruits will therefore, in all probability, result in a similar production of pectin and pectinic acids of varying composition. Sucharipa considers neutral pectin ('free pectin') to be soluble in water and the pectinic acids entirely insoluble. The pectinic acids, however, have not been shown to be insoluble : in fact, there is reason to believe that they are actually soluble. Hence, although, in the early stages of development of the fruit, neutral pectin alone may be formed, as is shown by the percentage of methyl alcohol in the extracted fraction, it seems most probable that in later stages of ripening, as the more stable pectose constituents are decomposed (see p. 828), pectinic acids will also appear. This point, however, can only be decided by a series of methyl alcohol determinations on the soluble pectic products which are produced from pectose during the development of the fruit. In the meantime, it is advisable to regard pectin as the primary decomposition product of pectose and the presence of pectinic acids as due to the subsequent decomposition of the pectin thus produced.

The fact that the neutral octomethoxy pectin itself is observed to develop as the fruit ripens is of interest, since it implies either that it exists as such associated with the pectose in some obscure combination (for instance, with cellulose groupings), or that any cellulose groupings originally present in its constitution are replaced by methoxy groups. The latter alternative suggests fixing of any methyl alcohol produced as a by-product of metabolism, and methyl alcohol has certainly been found to be widely distributed in extracts from many different plants (Haas and Hill, 'Chemistry of Plant Products', vol. i, p. 386, 1921).

A large amount of evidence therefore exists which suggests that pectose is a compound of cellulose and substances of pectic nature.

The work of Wiesner (37), Mangin (23), Tromp de Haas and Tollens (32), and Fellenberg (17) supporting this conception has already been referred to, and the more recent researches of Sucharipa afford

conclusive evidence. It seems probable, therefore, that the compounds known as 'pectocelluloses', described by many writers, are identical with pectose or protopectin, and the following observations serve to establish the identity of these plant substances. Cross (1895) (10) describes pecto-celluloses as substances giving rise to pectic acid and free cellulose on treatment with dilute alkali, and therefore regards them as compounds of pectin and cellulose. Cross and Bevan (1918) (11) state that the pecto-cellulose in flax and hemp fibres is hydrolysed by alkali, giving 20 per cent. pectic substance and 75 per cent. cellulose.

It follows, therefore, from the foregoing account of the main lines of investigations on the pectic constituents of the tissues, that accurate know-ledge of their properties and constitution is scanty. The importance of these substances as structural elements of plant tissues, and their relation to the economic problems already referred to (pp. 811 and 812), demand a more systematic and detailed investigation of their properties and constitution.

The following account deals with quantitative methods for the extrac-tion and estimation of the pectic compounds in fruit.[1] The methods have been applied to the study of the changes which occur in the pectic con-stituents of apples, and to trace their significance in all stages of normal metabolic drift, from early development to the ultimate death of the tissues.

General Account of the estimation of the Pectic Constituents of Apple-tissue.

In the following account the pectic constituents are provisionally described according to a classification based on the evidence already alluded to—Mangin (23), Fellenberg (17), Sucharipa (29)—and as a result of work carried out by the author (7–9).

I. *Pectose*—the water-insoluble pectic substance Carré (1925) located in the cell-walls in intimate association with cellulose. There is no reason to suppose that pectose is necessarily a homogeneous substance. It is possible that it is a complex formed by condensation of pectin and cellulose in varying proportions, and that the resulting compound will vary in stability towards reagents used for their extraction according to the number of cellulose groups in the molecule. (See pp. 818 and 819.)

II. *Pectin* [2]—the soluble modification which arises from the decomposition of pectose, and is invariably found to develop in ripening fruits. Fellenberg regards it as a neutral substance containing eight methoxy groups.

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[1] Preliminary results have already been published: Carré and Haynes (1922), Carré (1922, 1925).

[2] The existence of pectin has been questioned by Tutin (1923) (35). Evidence against this view has recently been published: Carré (1925) (9).

III. *Pectinic acids.* This term is suggested for the various intermediate acids of slightly varying composition and properties between neutral octomethoxy pectin and true pectic acid. They are produced in fruits in the more advanced stages of ripening, probably as a result of the hydrolytic decomposition of pectose, and probably ultimately of pectin itself. According to Fellenberg and Sucharipa, the differences in these may be attributed to the varying number of methoxy groups which they contain.

IV. *Pectic acid.* The stable product of the hydrolysis of pectose, pectin, and pectinic acids is a chemical compound of definite properties and composition (see p. 818). Fellenberg regards it as an acid with eight free carboxal groups, resulting from the complete removal of methoxy groups from the original neutral pectin, and there is substantial evidence in support of this view.

The pectic substances comprising the middle lamella are of uncertain composition, and will therefore be alluded to in the following account as ' middle lamella pectic substance ', in order to avoid confusion in nomenclature. A certain amount of evidence has, however, been obtained in support of the view that they are complex substances containing residues of pectinic acids, pectic acid, or the calcium salts of these substances.

The method adopted for the estimation of the pectic constituents of tissues depends upon the conversion of the various modifications into pectic acid, in which form they are finally converted into an insoluble calcium pectate of uniform composition, and estimated as such. The method of estimation which has already been described in detail (7)[1] is carried out-in the following stages :

(1) *Hydrolysis with sodium hydroxide,* whereby the pectin and any members of the pectinic acid series are converted into a soluble sodium salt of pectic acid.

(2) *Acidification with excess of acetic acid* in order to dissolve out the bulk of the impurities present in the solutions to be estimated.

(3) *Precipitation with calcium chloride* of a completely insoluble calcium salt of pectic acid. The precipitate is filtered off and boiled with water till free from chlorides and then dried at 100° C. and weighed.

It has already been pointed out that the pectin developed during the ripening of fruits, at any rate as ripening reaches an advanced stage, is not an individual substance but a mixture of neutral pectin and pectinic acids of varying methoxy content. This fact, however, will not affect the end-result

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[1] The accuracy of this method as compared with the alcohol method of estimation is fully discussed in this paper (Carré and Haynes (7)).

of the estimation, since the process of soda hydrolysis will convert all these modifications of pectin into the sodium salt of true pectic acid, as well as any neutral pectin itself which may be present.[1]

The accuracy of the above method of estimation necessarily depends upon the production of a definite chemical compound of constant composition—calcium pectate—and that there is such a compound has been proved by numerous chemical analyses carried out on pectic compounds derived from various sources. The calcium content of a number of samples of calcium pectate was determined (for method see (7)), and the results vary between very narrow limits—i. e. from 7·50 per cent. to 7·80 per cent. calcium, according to the purity of the original solution. It may, therefore, be regarded as an established fact that the calcium pectate obtained by the above method of estimation is a definite chemical compound of constant composition, identical with that obtained by Fellenberg by hydrolysis of the insoluble pectose in plant tissues. The constant composition of the calcium pectate justifies the use of this method for quantitative estimations of pectin, pectinic acids, and of pectic acid itself, and incidentally serves to demonstrate that the use of excess of acetic acid removes the greater part of the impurities, both organic and inorganic, present in the extracts. Small traces of inorganic material have been detected in the ash, notably iron, and these constitute the principal source of the impurities carried down with the calcium pectate precipitated from apple-juice. The error introduced into the weight of the calcium pectate by inorganic material will of course be very small.

The method, however, has been criticized as unreliable (Tutin (35)) on the ground that the procedure ' represents as pectin any acid which might happen to be present which yields a calcium salt insoluble in dilute acetic '. In other words, Tutin considers that acetic acid cannot be regarded as an efficient agent for dissolving out the impurities in the extract, and that the alcohol precipitation method is more accurate. Abundant evidence exists, however, that this is not the case, since a calcium pectate of uniform composition is invariably obtained by the use of this method,[2] except in the case of plant tissues containing oxalic acid and oxalates, and the more rarely occurring racemic acid or its calcium salt.

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[1] It is important to note that the ' soluble pectin ' alluded to in previous communications by the author and others is not therefore necessarily of uniform composition, but must be regarded as a collective term indicating the mixture of neutral pectin and pectinic acids which arises from the various forms of pectose in the tissues. In order to avoid confusion, it is advisable to adhere to the term ' pectin ' as applicable only to the neutral form and to use ' pectinic acids ' to denote the various forms intermediate between pectin and pectic acid.

[2] Small quantities of an insoluble substance have been isolated from apple-juice which preliminary investigation suggests may be a salt of dihydroxy-tartaric acid. The presence of this substance gives slightly higher results by the calcium pectate method of estimation than are obtained from similar material previously purified before estimation. In any case the alcohol precipitation method gives far more *inaccurate* results (see (7), p. 64).

The acids which occur widely in plant tissues are mainly tartaric, racemic, malic, malonic, succinic, citric, and oxalic acids, oxalic and tartaric acids usually occurring in the form of their calcium salts. Of these, only calcium oxalate and racemate are insoluble in water or in dilute acetic acid. The calcium salts of the remaining acids are soluble in acetic, while the salts of malic, succinic, and malonic acid are only insoluble in water if very concentrated. In carrying out estimations on plant extracts which contain insoluble acids or their insoluble salts, their insolubility in acetic acid has been overcome by a modification of the usual method which has been developed in conjunction with Miss A. M. Emmett.[1] It has been found that pectin can be quantitatively precipitated by alcohol containing hydrochloric acid, in which these insoluble plant constituents are readily soluble. The pectic acid resulting from the acid treatment, being difficult to manipulate as such, is dissolved in soda, and the usual method completed by addition of acetic acid and calcium chloride.

General Account of the extraction of the Pectic Constituents from Apple-tissue.

In order to follow out the seasonal changes in the pectic constituents of apples, and to investigate their relationship to one another, it was necessary to establish a quantitative method for their extraction. The method by which all the four classes of pectic compounds can be isolated depends upon the different chemical properties which each exhibits.

(1) *Extraction of pectin and pectinic acids.* A known weight of apple tissue is employed, and the pectin and any soluble pectinic acids are first removed by washing with cold water, and pressing in a small hand press (7 and 8). It is not possible to reach a stage when no further soluble pectic substance is removed, since the cold water apparently effects extremely slow but continuous hydrolysis of the pectose in the tissues.[2] A point is ultimately reached, however, when the fractions contain only negligible quantities, and sufficiently accurate estimates of the soluble pectic material initially present in the tissues can be obtained by discontinuing the operations at this stage (Carré, 7). The accompanying Table I illustrates the order of agreement when equal weights of uniform material are extracted for soluble pectic constituents. The resulting solutions are estimated in the usual manner by the calcium pectate method. The material used in this experiment was obtained from Cox's Orange Pippins (7).

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[1] Details of this investigation will shortly be published.
[2] This point is fully discussed in a separate paper (9). See also pp. 819 and 820 of the present paper.

TABLE I. *Extraction of Soluble Pectin and Pectinic Acids.*

Percentage weight of soluble pectin from five 100-grm. samples of uniformly mixed apple tissue.

$$\%$$
$$0·51$$
$$0·49$$
$$0·54$$
$$0·50$$
$$0·49$$

Mean 0·506

(2) *The extraction of pectose.*   The insoluble pectose constituent of the tissues is next removed by converting it into soluble pectic compounds by hydrolysis with dilute acid.   The process depends upon boiling a known weight of apple pulp with M/75 hydrochloric acid.   Since the removal of pectose from the sample used for the extraction of the soluble pectic material would necessarily involve considerable inaccuracy, a fresh sample of similar material is used, the amount of pectose actually present in the tissues being obtained by deduction of the soluble pectic material from the estimated total.   The use of stronger acid and higher temperatures (i.e. methods involving the use of an autoclave) is inadvisable, since further decomposition may be produced by such drastic treatment, resulting in the production of break-down products of pectic acid (see p. 819).   The pulp is washed to remove the bulk of the natural acids and soluble pectic material and is then boiled with the acid under a reflux condenser for successive three-hour periods.   The extract is filtered off, the residue washed with water to free it from the pectic material thus produced, and the process repeated till no further quantities can be extracted.   Boiling for longer periods than three hours should be avoided, since the pectic compounds thus brought into solution either tend to decompose or to become altered in physical state in some way which makes subsequent manipulation difficult.

Three to five such treatments are usually sufficient to remove all the pectose.   Aliquot portions of the total fractions obtained by extraction with hydrochloric acid and by the preliminary water extraction are hydrolysed with soda and precipitated in the usual manner with acetic acid and calcium chloride.

The accuracy of this method of extraction was investigated by treating ten 50-grm. samples of uniform apple tissue.   A specimen set of results is given in the following table.   The material used for the experiment was obtained from Australian cold-stored apples.

TABLE II.

Weight of Pectose obtained from 50-grm. Samples of Uniform Apple Tissue (fresh) estimated as Calcium Pectate.

Grm. calcium pectate.

0·76
0·77
0·77
0·75
0·76
0·74
0·76
0·73
0·77
0·73
—
Mean 0·75

The divergences from the calculated mean of these results is not considerable, and may be attributed either to errors arising from the method of extraction or to the difficulty in mixing the material thoroughly. In order to settle this question, the experiment was repeated on dried, ground, homogeneous material, obtained by drying apple pulp, similar to that used in the preceding experiment, for thirty-six hours at 100° C.[1] Weighed samples of this material were extracted as before with M/75 HCl. The figures given in the accompanying table show that the uniformly mixed material gives results which agree closely.

TABLE III.

Weight of Pectose from 100 grm. of Dried Apple Tissue.

Grm.

0·75
0·77
0·77
0·76
0·75
0·77
0·75
0·76
0·77
0·77
—
Mean 0·76

It may therefore be concluded that the variations in the first experiment are due to the difficulty in mixing fresh material thoroughly. This

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[1] This method has been found to give a reliable value for total dry weight, and has apparently very little effect on the total of the pectic material estimated subsequently (1). Sucharipa (29), however, considers, as a result of his methyl alcohol determinations on fresh and dried material, that a slight conversion of pectose into soluble pectic substances does take place, but shows that the total pectic material as estimated by methyl alcohol is unaltered.

difficulty may be attributed to the enormous differences between individual apples of the same batch, and to variability in the distribution of the pectic material in the different regions of the apple. The importance of this factor is shown by the following set of pectose estimations carried out on each of ten *individual* apples, similar to those used in both the preceding experiments. The mean of the ten results agrees well with that of the other two sets, but there is a large individual variation which accounts for the difficulty in mixing the cut-up apple tissue.

TABLE IV.

Variation in the amount of Pectose in Individual Apples.

%
A.	0·69
B.	0·75
C.	0·69
D.	0·84
E.	0·79
F.	0·81
G.	0·74
H.	0·77
I.	0·68
J.	0·72

Mean 0·748 [1]

(3) *Extraction of middle lamella pectic substances.* Microscopic examination of the residue at this stage, by staining with ruthenium red, reveals the presence of substances of pectic nature still present in the region of the middle lamella, although all traces of stainable material have disappeared from the cell-walls themselves. The pectic constituents of the middle lamella must therefore be insoluble in the water and dilute acid used for the removal of pectin, pectinic acids, and pectose, which suggests that they consist of a far more complex combination than the other pectic constituents.[2] A certain amount of evidence exists that the middle lamella pectic substances are in some way combined with calcium. Molisch (25) observed that, after treatment of sections with dilute sulphuric acid, crystals of calcium sulphate appeared all along the lines of the middle lamella. Also in the course of the work already referred to calcium oxalate crystals were detected in a similar position to those observed by Molisch after treatment with ammonium oxalate and hydrochloric acid.

[1] These results were repeatedly confirmed, and there seems no doubt that the correspondence was valid for the apples used. On the other hand, later experiments carried out by Miss A. M. Emmett suggest that differences may arise between fresh and dried material under other circumstances. This point is being further investigated.

[2] A detailed microscopic investigation has been carried out by the writer in conjunction with Dr. A. S. Horne on the localization and distribution of the pectic substances in apple tissue. An account of this work will appear shortly.

Any salts will, however, be decomposed by the hydrochloric acid used in the process of extraction of pectose.

It was decided to try the effect of extraction with sodium hydroxide, after the acid treatment for the removal of pectose, according to a method for middle lamella solution used by Mangin (24) with successful results. The residue after extraction of pectose, pectin, &c., was therefore boiled with $M/75$ NaOH for successive half-hour periods. Stronger alkali was avoided, as this produces decomposition of pectic acid into the lower acids already referred to. It was found that two such extractions served to remove all remaining traces of pectic substance from the tissues, and that the cells had become completely separated from one another, owing to the solution of the middle lamellae. The alkaline extract thus obtained was treated with acetic acid and calcium chloride, and the resulting precipitate was found to have an ash corresponding to the 7·60 per cent. of the normal calcium pectate ; the alkaline extract therefore presumably contained sodium pectate in solution. There are, therefore, considerable grounds for regarding the middle lamella as consisting of complexes containing residues of pectic or pectinic acids. Too little is yet known to form a definite hypothesis, but it is possible that these middle lamella compounds are akin to pectose in that the *whole* of the carboxyl groups are replaced by cellulose. Sucharipa emphasizes (see pp. 819 and 820) the stability of the pectose fraction which contains the least methoxyl and most cellulose groupings replacing the carboxyl, and obtains his final fraction by means of ammonium oxalate, the methoxy content of the resulting pectic acid corresponding to one methoxy group. The present writer has found it impossible to remove the middle lamellae with ammonium oxalate, and that the addition of hydrochloric acid followed by boiling with sodium hydroxide is the only possible way of entirely dissolving out the residual pectic substance of the middle lamella as detected by ruthenium red. These conclusions suggest that the pectic constituents of the middle lamella complex may be pectic acid in which all the carboxyls are replaced by cellulose.

It has not been definitely established that the above-described method is absolutely quantitative, especially in view of the fact that microscopical investigations reveal considerable differences in the behaviour of the middle lamella. In any case, the method is satisfactory for extracting the pectic constituents of the middle lamella and for obtaining a relative measure of the extent to which they occur in the tissues.

Treatment of the cell remains with Schweitzer's reagent causes solution of the cellulose, and the sections consequently disappeared except for minute traces of material probably of a lignified nature.

The accompanying diagram illustrates the relation of the processes of extraction and estimation of the pectic constituents produced naturally in the apple or chemically by the use of reagents.

Diagram illustrating the Method of Extraction and Estimation of the Pectic Constituents of Apples.

Pectose
↓
Pectin→Pectinic acids→Pectic acid→Sodium→Calcium
                                      pectate   pectate

Pectic substances
of the
middle lamellae.
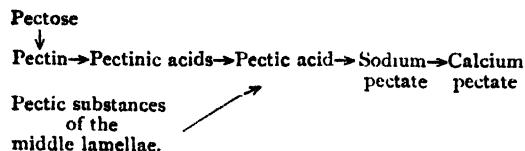
TABLE V.

Giving Amounts of Pectic Constituents in Apple Tissue of Bramley's Seedlings (stored 1° C., October 1923–August 1924, Low Temperature Station, Cambridge).

Time in Weeks.	A. Soluble Pectic Material.	B. Total Pectose and Soluble Pectic Material.	C. Pectose B−A.	D. Middle Lamella Pectic Constituent.	E. Total Pectic Constituents A+C+D.
1st	0·00	0·74	0·74	—	—
3rd	0·01	0·75	0·74	0·28	1·03
4th	0·03	0·74	0·71	—	—
8th	0·08	0·75	0·67	—	—
10th	0·09	0·73	0·64	0·29	1·02
17th	0·14	0·74	0·60	0·28	1·02
21st	0·16	0·72	0·56	—	—
23rd	0·16	0·75	0·59	0·26	1·01
26th	0·19	0·84	0·65	—	—
29th	0·16	0·84	0·68	0·20	1·04
32nd	0·19	0·85	0·66	0·19	1·04
36th	—	0·77	—	—	—
40th	0·19	0·72	0·53	0·19	0·91
45th	0·21	0·73	0·52	0·18	0·91
49th	0·21	0·70	0·49	0·18	0·88

The Seasonal Changes in the Pectic Constituents of Apples.

As a result of this work on the extraction and relative estimation of the pectic constituents of apples, it has now become possible to apply the method above described to a study of the inter-relationships of these compounds and to follow the changes which they undergo during the development and senescence of the fruit. Preliminary investigations have been carried out on Bramley's Seedlings from the early unripe conditions of the fruit to the latest stage which can be maintained in cold storage at 1° C. The estimations were made fortnightly on 50-grm. samples taken from ten apples cut up and thoroughly mixed. The accompanying series of graphs illustrates the relative changes in the pectic constituents of the samples as observed by chemical estimations.

(a) *Changes in pectin.* It has already been shown (Carré (8)) that the ripening of apples is associated with a development of ' soluble pectin '[1] in

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[1] See note on page 823 concerning the use of the term ' soluble pectin '.

the expressed juice. At the normal time of gathering apples the amount of soluble pectic material extracted is negligble, but as ripening proceeds it gradually increases (Fig. 1, A).[1] The rising portion of the curve immediately preceding the maximum pectin content corresponds to the fully ripe state of the fruit when it is in its prime from the market point of view.

The pectin content of the fruit was maintained at this high level for about six months in cold store ; at this point the experiment was concluded, but results obtained from similar experiments carried out with apples show that a steady decrease in pectin is subsequently observed (Carré (8)), probably as a result of decomposition by hydrolysis or of oxidative processes in the tissues. This period of falling content of soluble pectic material corresponds to the increasingly soft and over-ripe condition of the fruit known to the trade as ' mealiness '.

(b) *Changes in the pectose constituents.* Estimations of pectose carried out simultaneously show (Fig. 1, B and C)[2] that the pectose decreases in amount as the season advances (1st–17th weeks). This decrease corresponds to the increase observed in the soluble pectic constituents of the tissues and is readily explained by the fact that pectose is converted during ripening into pectin and pectinic acids.

As the fruit becomes over-ripe, the relationship between pectose and pectin becomes more obscure. The steady production of soluble pectin in over-ripe fruit suggests that a simultaneous decrease in the pectose might be expected. It will be seen, however, that the reverse case holds, namely, the pectose appears to increase markedly for a period of eight to nine weeks from March to May (Fig. 1, B and C). The only possible explanation is that the supply of pectose is augmented from another source. It will be seen that the changes in the pectic substances of the middle lamella suggest a possible source of this supply.

In the more advanced condition of senescence the pectose content of the samples falls rapidly. The stock of apples used for these experiments was exhausted before the senescent condition became very marked, but complete disappearance of pectose has been observed in other samples of apples examined in the last stages of senescence.

(c) *The changes in the pectic substances of the middle lamella.* Throughout ripening, the middle lamella pectic substance maintains a

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[1] The values for soluble pectin materials (Fig. 1, A) are only approximate. It was ascertained that the soluble pectin in the expressed juice was proportional to the total amount of soluble pectin material as determined by exhaustive extraction of the apple tissue with cold water and pressing. Hence, as time did not permit of such lengthy processes, the soluble pectin content of the juice only was estimated.

[2] The figures plotted on Fig. 1, B, represent pectose and in addition any soluble pectic material present at the time of the experiment. The actual amount of pectose can be readily calculated by deducting the weights of soluble pectic material previously determined from separate samples of material (see pp. 824 and 827).

THE VARIATIONS IN THE PECTIC CONSTITUENTS OF APPLES
Bramley's Seedling Cold Stored at 1°C, 1923–1924 *(Cambridge Low Temperature Station)*

A. Soluble Pectic Material (estimated in juice)
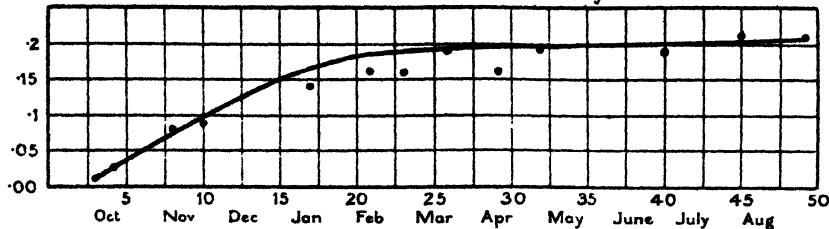
B. Total of Insoluble Pectose & Soluble Pectic Material (H₂O & HCl extraction)
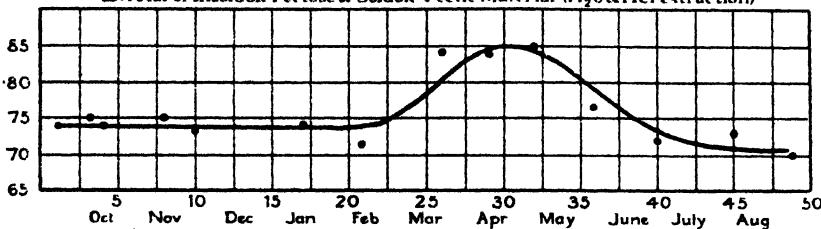
C. Insoluble Pectose (calculated from difference between A & B)
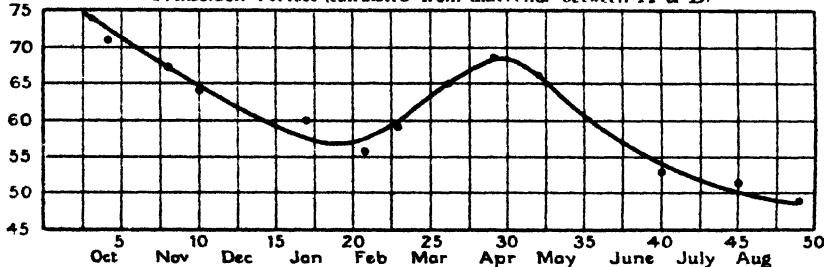
D. Pectic Substances produced by Solution of the Middle Lamellae (HCl & NaOH extraction)
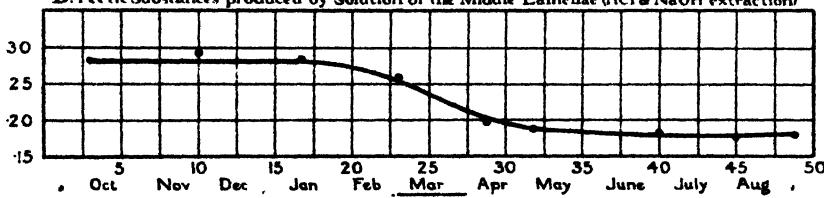
E. Total Pectic Constituents (Calculated from sum of estimations B & D)
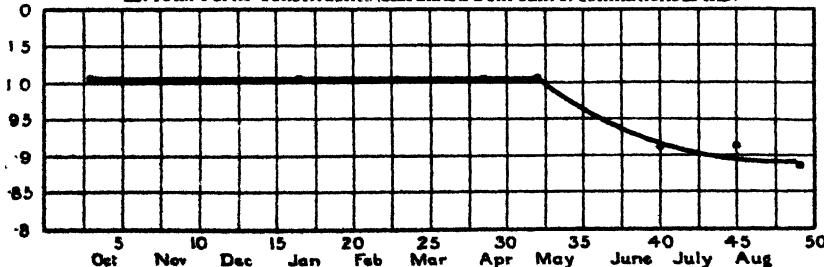
FIG. 1.

constant level, but when the soft, over-ripe condition of the fruit becomes apparent, the amount begins to decrease rapidly (Fig. 1, D). This process of degradation continues till in the last stages of senescence only a negligible amounted is extracted from the tissues. Microscopic observations show that this intimate association of the decomposition of the middle lamella constituents with the over-ripe condition of the fruit is marked by a gradual loosening of the cells, till ultimately in the most advanced condition of over-ripeness the cells are entirely separated from one another, owing to complete disappearance of the middle lamella substance.

The decrease in the pectic constituents of the middle lamella offers a possible explanation of the apparent increase in pectose which occurs simultaneously (Fig. 1, B and C). The decrease in the middle lamella substances approximately corresponds to the increase in pectose, and it is conceivable that there is some connexion between these observed facts. It may be suggested that in the over-ripe condition of the apples the middle lamella undergoes decomposition and its pectic components gradually pass into a less stable form which is estimated along with the pectose constituents of the tissues.

The Significance of the Changes in the Pectic Constituents.

The foregoing account of the changes in the pectic constituents of apples suggests that these compounds play an important part in the metabolic processes during development, and that they are intimately associated with the gradual disintegration of the tissues which accompanies the senescent state.

Experimental observations show that pectose, pectin, pectinic acids, and the middle lamella substances undergo a series of interrelated changes which ultimately result in their decomposition. These changes may be conceived to take place as follows : The insoluble pectose is either resolved into soluble neutral pectin or into pectinic acids, both products being of less complexity than the original pectose. The pectic compounds thus produced apparently remain *in situ*, merely replacing their insoluble precursor. There seems no necessity for supposing, as many writers do, that the pectin finds its way into the cell sap; neither is there any evidence that this is the case. This view presumably arose from the observation that when the tissues are pressed mechanically the expressed cell sap carries away with it any pectic substance existing in the cell-walls in a soluble condition.

The disappearance of pectin and pectinic acids observed in over-ripe fruit may be attributed to a process of decomposition similar to that produced artificially by hydrolytic agents. The neutral pectin probably breaks down into the pectinic acids with varying methoxy content described by Fellenberg, ultimately giving rise to pectic acid itself with no methoxy groups. It is then possible that the simpler acids described by Ehrlich are

eventually produced from pectic acid, in which case galactose, arabinose, and methyl pentose will be set free (see pp. 818 and 819), as is the case when pectic acid is decomposed by powerful artificial hydrolysis. It is possible that the carbohydrate constituents of the pectic acid molecule are subsequently utilized in respiration. This decomposition of pectic acid will therefore result in a fall in the total pectic constituents, since their method of quantitative estimation depends on their conversion into pectic acid as such (Fig. 1, E, 33rd–49th week).

The pectic constituents of the middle lamella also undergo decomposition and solution as the development of the fruit proceeds, but the process cannot be interpreted till more information has been obtained of the precise nature of the middle lamella pectic compounds. The foregoing evidence suggests that they are partially resolved into pectinic acids, pectic acid, or compounds closely akin to these, in which case any conditions capable of bringing about the decomposition of the pectic acid produced from the other pectic constituents of the cell-walls would consequently involve the decomposition of the pectinic or pectic acids produced from the disintegration of the middle lamella, and similar cleavage products would arise from both sources.

Taking into consideration the observations stated above and the results obtained by Ehrlich, Fellenberg, and other investigators, the metamorphoses of the pectic substances may be provisionally represented as follows:

Diagram representing the Pectic Changes in Apples.

Pectose

Cellulose   Pectin

Methyl alcohol   Pectinic acids

Methyl alcohol   Pectic acid

Galactose-galacturonic acid   Pentoses   Hexoses

Galacturonic acid
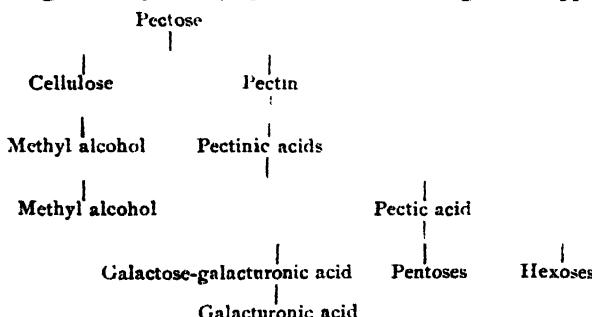
Microscopical observations made periodically on the tissues bear out the conclusions arrived at from purely chemical studies. It was observed that as the season advances the walls get thinner and contain less pectose material, and that the soft, over-ripe condition occurs simultaneously with a gradual separation of the cells owing to the disappearance of the middle lamella pectic substance. In the last phase of physiological death, still more characteristic phenomena are observed. The cells become entirely separate from one another, the cell contents plasmolyse, and here and there are observed to have disappeared entirely.

3 K

In the last stages of senescence the pectic substances are found to have almost entirely disappeared, but the outline of the cells is still maintained by the cellulose constituent of the walls.

The commencement of the over-ripe state is plainly discernible by the pectic disturbances as observed microscopically ; in fact it may be stated with a considerable degree of assurance that the stage of maturity of an apple can be ascertained by microchemical examination of the tissues alone.

The Mechanism of the Pectic Changes in Apple Tissue.

The association of the pectic metamorphoses with the metabolic drift of the apple is obviously of the greatest importance ; at this early stage in the work, however, it is uncertain whether the decomposition of the pectic constituents is the primary cause of the death of the tissues in that it brings about the separation of the cells and disintegration of the cell-walls, or whether it is a secondary effect consequent upon certain chemical changes in the cell contents, attendant upon the senescent state of the fruit.

A. *Conversion of pectose into pectin during ripening.* The conversion of pectose into the form of soluble pectin during the ripening of fruits was attributed by Frémy solely to the action of plant acids, but later workers recognized the possibility of enzyme activity. A certain amount of experimental evidence has been obtained which suggests that a specific enzyme, propectase or pectosinase—Erfurt (1904) (15), Thatcher (1915) (30), Atkins (1916) (2)—is responsible for the decomposition of pectose.

As a result of preliminary work carried out by the writer, evidence that enzyme action is an important factor in the hydrolysis of pectose has been afforded by the fact that the rate of development of soluble pectic substances by treatment with water at 45° C. is markedly greater than that observed when the tissues are submitted to the action of boiling water. Presumably the lower temperature is favourable to the action of the enzyme, whereas the higher temperature destroys its activity, and the decomposition of the pectose consequently depends upon its hydrolysis by the water alone.

Similar results were obtained by extracting the bulk of the soluble pectin from ripe apples, and leaving the residue in a moist air chamber for forty-eight hours. A large amount of pectin was found to have developed on subsequent extraction with water, whereas a similar sample, previously heated for one hour at 100° C., exhibited no such marked increase in pectin.

It follows, therefore, that enzyme activity probably plays an important part in the natural decomposition of pectose, and this factor must be taken into consideration when methods which are not detrimental to the activity of the enzyme [1] are employed for the extraction of the soluble pectic constituents of tissues.

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[1] See The Relation of Pectin and Protopectin in Plant Tissues (9).

B. *Decomposition of pectin and the pectic constituents of the middle lamella during senescence.* The possibility of enzyme activity in bringing about the decomposition of pectin, pectinic acids, and the solution of the middle lamella substances in over-ripe fruits has been investigated by several workers, but very little experimental evidence has as yet been obtained. Frémy (1840) first described an enzyme which he calls ' pectase ', and which he considers responsible for the development of insoluble pectic acid from pectin. Further researches on ' pectase ' were carried out by Bertrand and Mallèvre (1894–5). They found that the activity of the enzyme depended upon the presence of calcium, whereas its action was inhibited by free acid, a concentration of 0·1 per cent. hydrochloric acid being sufficient to entirely check its activity. Similar results were obtained by Bourquelot and Hérissey (1898–9) (4), who also isolated a second enzyme ' pectinase ' from germinating barley, which was capable of converting both pectins and pectic acid into a mixture of reducing sugars. Confirmation of Bourquelot and Hérissey's work is afforded by the researches of Bridel (1905), and more recently by Euler and Svandberg (1917–19) (16), but very little accurate information has as yet been obtained of the mechanism of pectic decomposition in plant tissues. Various investigations on the effect of bacterial and fungal attack on plant tissues throw considerable light on the problem. Winogradsky (1895) (39) pointed out that the retting of flax and hemp fibres was due to decomposition of the pectic cell cement by bacteria, and attributes this so-called ' pectic fermentation ' to enzymes secreted by the bacteria themselves. The effect of fungal invasion on the pectic substances in the tissues has received more attention. In 1886 de Bary (13) observed that the fungus *Peziza sclerotiorum* caused death of plant tissues by secreting a substance which effected partial disintegration of the cell-walls. Extracts of the diseased tissue caused a similar disintegration of the healthy tissue of carrots, but if boiled before innoculation no effect was produced.

Marshall Ward (1888) (24) observed that various species of *Botrytis* had a similar effect on healthy plant tissue. Both investigators attributed the disintegration of the tissues to the presence of enzymes in the digestive juices of the invading fungi.

A detailed investigation on the mode of fungal attack on plant tissues was published by Dr. W. Brown (6). The author describes a method of obtaining a powerful enzyme extract from the germ-tubes of *Botrytis cinerea*. The action of this extract on various plant tissues (potato, turnip, beet, apple, &c.) was found to operate in three stages, according to the time of action :

    (1) Solution of the middle lamella.

    (2) *Partial* disintegration of the cell-walls.

    (3) Death of the cells.

Brown observed that complete loss of cohesion of the tissues by solution of

the middle lamella could be obtained without affecting the remainder of the cell-walls, in which case the cell maintained its normal living condition. Continued action of the extract, however, resulted in partial disintegration of the cell-walls and simultaneous death of the protoplasm. In no case did Brown observe complete solution of the cell-wall. This observation is readily interpreted by assuming that the enzyme is only capable of acting on the pectic compounds of the cell-wall and middle lamella, and not on the cellulose components of the tissues.

Similar results were obtained with the fungal extract as by inoculating sections of the tissues with the fungus itself, and Brown therefore concludes that ' all the macerating and lethal effects of the fungus can be explained on the basis of the properties of the standard lethal extract '.

The activity of the extract was found to be retarded by temperatures of 55° C., and complete deactivation was obtained above 65° C.

Brown therefore concludes from his observation that the action of the fungal extract is due to the presence of an enzyme, which he regards as identical with the so-called ' cytase ' or ' pectinase ' of earlier workers. Confirmation of Brown's work is afforded by a series of investigations on the mode of attack of *Sclerotinia cinerea* (Valleau (36)). Valleau showed that the fungal hyphae caused solution of the middle lamella of apple tissue, and that an extract of rotted apple had a similar effect on healthy tissue. He attributes the solvent action to an enzyme secreted by the hyphae of the fungus, and shows by a series of photomicrographs that it is secreted in advance of the hyphae.

Willaman (1920) (38) observed that, if *Sclerotinia cinerea* was grown in pectin solution as its source of nutriment, the pectin was converted into an insoluble gel of pectic acid, and that, ultimately, reducing sugars were split off from the pectic acid and assimilated by the fungus.

A series of investigations has been carried out by the present author, in conjunction with Dr. A. S. Horne, on the changes produced in the pectic constituents of apple tissue as a result of fungal disease, by comparing sections of normal tissue stained with ruthenium red with infected tissue similarly stained. In all cases examined, pronounced pectic disturbances were detected, entirely similar to those obtaining in normal senescence of the tissues (see pp. 830 and 831).

It may therefore be concluded, from the evidence already existing, that the disintegration of plant tissues produced by fungal diseases, and the similar phenomena observed in normal physiological death, may be attributed to a series of changes in the pectic constituents which are controlled either by enzymes which occur in the living tissue or by enzymes secreted by the fungi in the case of outside infection.

The facts that the susceptibility of the apples to fungal disease varies with the condition of the maturity of the fruit, and that certain kinds of

apple are much more readily attacked than others, suggest that the chemical composition of the fruit exercises a controlling effect on the activity of the enzyme producing the degradation of the pectic constituents of the tissues. It is significant that the acceleration in the decrease in the acidity as determined by estimation of the malic acid [1] (see Figs. 1, D, and 2) occurs at the same time (approximately the 28th week) as the onset of the break-

The Malic Acid Content of Apples (calculated from titration of juice)
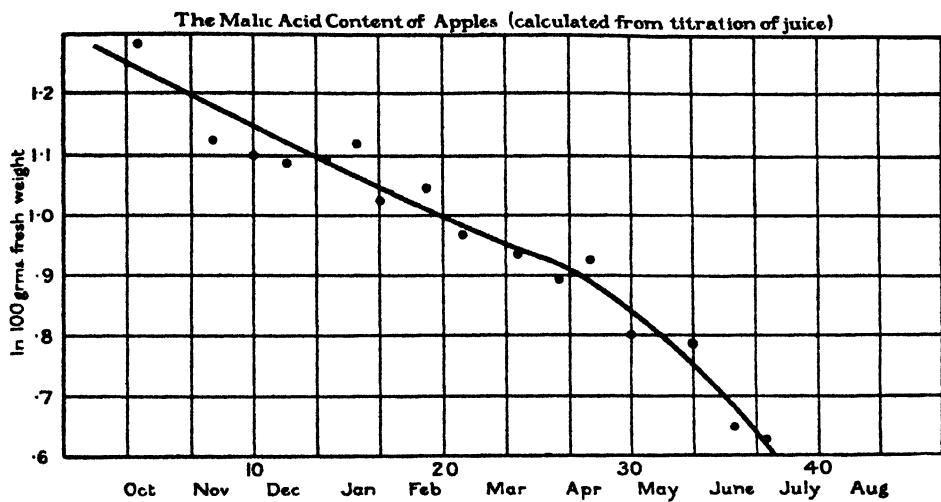
Fig. 2.

down of all the pectic constituents of the tissues is observed. This fact suggests that the enzymes effecting the decomposition of the pectic acid produced from the hydrolysis of the other pectic substances in the tissues can only operate when the acidity of the cell sap is lowered.

This preliminary evidence suggests that the composition of the fruit is intimately associated with its keeping properties, and with its susceptibility to attack by fungi and bacteria, but the whole problem is in process of a much more detailed investigation.

[1] The acid determinations were carried out by Dr. Haynes.

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The Correlative Inhibition of the Growth of Axillary Buds.

BY

R. SNOW.

With four Figures in the Text.

INTRODUCTION.

WHEN the growing apex of the shoot of a leguminous seedling is cut off, or prevented from growing, one or more of the axillary buds already formed lower down grow out and form new shoots. In many other kinds of seedlings of monopodial growth, the resting axillary buds can be made to grow out by removing the apex.

Since it can be shown that it is not any 'wound-shock' which makes the axillaries grow out (9, p. 242 seq.). it follows that it is the original apex of the shoot which, while present, must have been preventing the axillaries from growing. The problem is, therefore, not why do the axillaries grow out after decapitation, but what is the nature of the influence exerted upon them by the apex which prevented their growing out before, a point of view clearly expressed by McCallum (9, p. 256-7).

It is possible that this influence may be only indirect : it is possible, that is to say, that the apex when present may be influencing some other part of the plant in such a way that the latter inhibits the axillaries. This point of view should appeal more to those who prefer to think of a 'form equilibrium' which is upset by removal of the apex. For it will still be necessary to suppose that during the 'equilibrium' the apex is somehow influencing the rest of the plant, since otherwise its removal could not make any difference. Thus, however the problem may be stated, the essential point will still remain, that the apex when present must exert some influence which directly or indirectly prevents the axillaries from growing out. It is the nature of this influence and the way by which it travels which it is here proposed to study. For simplicity, it will be spoken of simply as the inhibition of the axillaries by the apex.

As to the nature of this inhibition, the possible theories may be classified naturally, in accordance with Child and Bellamy (3), under three headings.

(1) It has been suggested that the growing apex somehow draws to itself the supply of nutriment, or some particular substance necessary for growth, and so keeps the axillaries starved and unable to grow.

(2) The inhibition might be due to an inhibiting substance formed by the growing apex and passed back down the shoot.

(3) It might be due to some physiological process of unknown nature initiated at the apex and transmitted back down the shoot.

The first of these, the 'nutritive exhaustion' theory, may at first seem simple and plausible, but is really, from a physico-chemical point of view, extremely obscure. It is extremely difficult to imagine any mechanism by which one part could draw to itself nutritive substances from another, so as to starve it. As pointed out by McCallum (9, p. 257), one would expect the nutritive substances to diffuse towards the regions of lowest concentration, and thus automatically to compensate any incipient local starvation. Moreover, the experimental evidence is strongly against the theory. For this, reference should be made to the numerous ingenious experiments of McCallum, which show to what an extent the first outgrowth of the axillary buds is independent of the prevailing nutritive conditions, and also to the results of Mogk, who in the course of many interesting experiments on the various manifestations of correlative inhibition was led decisively to reject all nutritive theories of its mode of working (10, p. 664). These two investigators do not attempt to decide between explanations (2) and (3).

An important advance was made by Child and Bellamy (3 and 4), who succeeded in interrupting inhibition in different organs of various plants, including the epicotyl of *Phaseolus*, by surrounding them with a coil containing water at 0° C. to 4° C. Their results would, as they point out, be difficult to reconcile with a 'nutritive' theory of inhibition. But when they also conclude from their experiments that inhibition is brought about by the transmission of some dynamic process, and not by the transportation of an inhibiting substance (4, pp. 263–4), this seems not at all certain. For the experiments only show that the transference of inhibition depends on the physiological activity of living cells, and the movement of inhibiting substances might itself depend on the activity of living cells.

Their conclusion is only made probable, and not completely proved, even for the inhibition of development of the runner-tip of *Saxifraga sarmentosa*, which, after being 'physiologically isolated' by the cold-water coil, must still have been supplied by the transpiration current. For the inhibiting substances, if there were any, may not have been able to enter the vessels. They may also have been unable to enter the vessels in the

experiments on the petiole of *Bryophyllum*, in which too, since the leaves were submerged, the movements of the transpiration current may have been very slight.

In the experiments on *Bryophyllum* another complication is that, by the coil round the petiole, the leaf may have been isolated not only from the growing points of the shoot, but also from the roots. Now, in some conditions at least, the rudiments in the notches of the leaves grow out if the roots alone are removed, even though the shoots remain (6, p. 146). Consequently it is not at once obvious whether the coil produces its effect by interrupting inhibition from the shoot or from the root.

Loeb also has published numerous papers on *Bryophyllum calycinum* (8), in which, besides various other questions (polarity, geotropism, &c.), he considers the problem of inhibition. He considers that his experiments support the ' nutritive exhaustion ' theory. These experiments are very numerous, but examination will show that all the relevant results, with only one exception, can be expressed by the following statement (similar to one given by Loeb as one of the ' rules of inhibition ') : that if one part (B) of a plant is prevented from regenerating by being left in connexion with another part (A), it transfers to A the same quantity of material as it would itself have expended in regeneration if completely isolated. From this Loeb concludes that it is because A is drawing the material to itself that it inhibits B.

But it is at once obvious that there is not the slightest reason for reading the causal series in this order rather than in exactly the opposite order. It can just as well be maintained that because A has first inhibited B and is thus making new growth while B is not growing, as a result A then is able to draw the material to itself.

Loeb's experiments are thus not critical: they will fit in with a ' nutritive exhaustion ' theory, but can equally well be explained on any other theory of inhibition. He has no doubt struck on various points of interest, but towards solving the problem of the mechanism of inhibition he has by these experiments contributed absolutely nothing.

He also reports, however, one quite different experiment, which is the exception referred to above (' Bot. Gaz.', lxiii, 1917, p. 244). Incisions were made into a leaf alternately from opposite sides, and much more than half-way through it. The leaf-tip was still inhibited though joined to the stem only by a long sinuous path through parenchyma. Loeb draws the surprising conclusion that this experiment supports the ' nutritive exhaustion ' theory. But it would be expected that nutriment would only move extremely slowly, if at all, by such a long parenchymatous path. Thus the experiment really tells heavily *against* his theory: it is not favourable, either, to a theory of transportation of inhibiting substances.

The experiments to be reported in this paper aim at throwing further

light in various ways on the nature of correlative inhibition in leguminous
seedlings. As a preliminary, it was thought desirable to determine through
which tissues of the stem it is able to act.

MATERIALS AND METHODS.

The experiments were carried out during the summer months, on seed-
lings of *Phaseolus multiflorus* and *Vicia Faba.* The plants were grown in
frames or in a glass-roofed part of the laboratory. They were found to
grow excellently in a mixture of half-and-half sand and sawdust.

The *Phaseolus* seedlings were usually operated on when the first inter-
node had begun to elongate, a stage which, at the usual temperatures of
60° to 70° F., was reached at about a fortnight from sowing. This usually
leaves, if big seed has been used, another eight or ten days before the
cotyledons become exhausted. The axillaries, therefore, had the chance of
growing out before the cotyledons were exhausted. This was thought
desirable, although, as McCallum has shown (9, p. 104 seq.), they will still
grow out slowly even without the cotyledons. So long as the plants are not
interfered with, the axillaries of the cotyledons hardly ever grow out. But
just occasionally (perhaps in one plant in a hundred) an axillary was seen to
grow right out for no apparent reason. The axillaries usually remain quite
small—less than 1 mm. long. Fairly often they grow to a length of 2 or
3 mm., but then, in intact plants, grow no more.

I. THROUGH WHICH TISSUES DOES INHIBITION ACT?

1. *Inhibition through a ringed zone.*

It was found possible to 'ring' the epicotyl of a *Phaseolus* seedling
without injuring the wood and pith, by stripping off with a thin, pointed
scalpel all tissues external to the wood. It is necessary to make sure that
the cambium is entirely removed, since even a small shred of cambium, if
left uncut, will soon start a vigorous regeneration. This can be made easier
by leaving the ringed zone for an hour or two to dry, and then re-examin-
ing for remains of cambium; but this practice was seldom found necessary.
The rings were finally coated with vaseline.

EXP. 1. *June* 1924.

Seven *Phaseolus* seedlings were 'ringed' down to the wood in the
epicotyl, the ringed zone being about 2–3 cm. long.

After eight days, axillaries had not grown out in any of them.

Five of these seedlings were now examined microscopically, and found
to show absolutely no remains of cambium nor regeneration outside the
wood. Within the woody cylinder, the outer cell layers of the pith had

begun to divide and form radial rows of large-celled parenchyma, but this newly formed tissue contained no elongated conducting cells, and therefore introduced no new factor into the situation. When, however, ringed plants are left for longer than this, a new process eventually starts by which, at various points in this tissue newly formed from the pith, the cells divide up and form new conducting strands. But in no case was this process found even to have begun until eleven days or more after the operation, so that it does not affect the present question.

Controls. The total number of *Phaseolus* seedlings that were decapitated in the epicotyl on various occasions is 57. Of these, 54 showed obvious outgrowth of one or both cotyledonary axillaries after 6 days at the latest. In 2 they delayed for 7 or 8 days before growing out, and in 1 only they failed to grow out at all. In the great majority the axillaries were obviously growing out after 4 days only.

These results show that inhibition can act through a ringed zone of the epicotyl.

In order to determine whether inhibition can act through a much longer ringed zone, the following experiment was carried out later :

EXP. 2. *September* 1924. Temp. 60°–65° F.

Five *Phaseolus* seedlings were ringed in the epicotyl for distances ranging from 6·5 to 8 cm.

After eight days, the axillaries of four of them showed absolutely no trace of growth. In the fifth, one axillary had reached 2 mm., but had then grown no more.

The cotyledons of these plants did not become exhausted for seven days or more.

Inhibition can therefore act through a ringed zone as long as 8 cm. Ringing was found not to prevent the growth of the main shoots above the rings ; indeed, they seemed to grow in length more rapidly than did the shoots of intact plants.

2. *Can Inhibition act through the Pith ?*

This question obviously cannot be answered by simply cutting through all the vascular tissues in a ring round the epicotyl, for then the parts above would perish. The experiment was therefore arranged as follows :

Seedlings of *Phaseolus* were carefully dug up, and their lower parts were completely split in halves by a longitudinal split in the median plane, starting at the base of the epicotyl and passing down between the cotyledons and out through the hypocotyl and main root. Next for a few centimetres upwards from the upper limit of the split, the woody cylinder of the

epicotyl was laid bare along two strips at the front and back, and then at front and back two straight narrow strips of the wood were prized up and cut away, so as to expose the pith. Finally, at the level of the top of these strips, the wood and external tissues of one side only (the 'A' side) of the epicotyl were prized up and cut through, the other side (the 'B' side) being left intact. The diagram (Fig. 1) may make the arrangement clearer.

As a result, the cotyledons and their axillaries were now situated upon different halves of the plant, which were connected together by the pith alone. Of these halves, the one (B) was still joined up by continuous wood and phloem with the shoot-apex, the other (A) was connected only by way of the pith. The exposed cut surfaces were vaselined. Those of the root soon hardened or callused over. The halved root systems were still able to function, for the upper parts of the plant remained fresh and continued to grow.

The result of the final series of experiments is given in the following table. The figures give the lengths in millimetres of the axillaries of the two different sides (A and B) of each plant, after the number of days stated above the columns.

FIG. 1. Diagram to illustrate Experiment 3. *s* indicates the strip along which cortex and xylem have been removed.
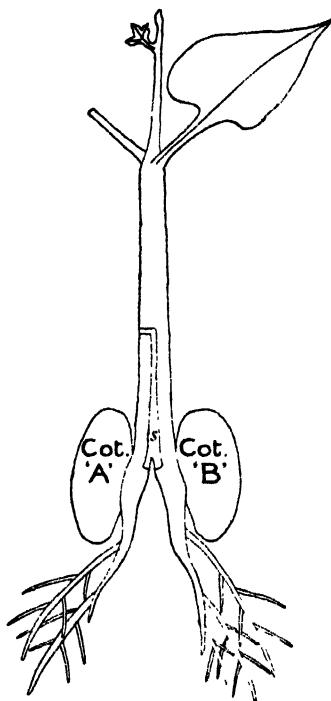
EXP. 3.   TABLE I.   *September* 1924.   Temp. 60°-65° F.   Outgrowth of axillaries in millimetres.

	6 *days.*		8 *days.*		11 *days.*	
	A axillary.	B axillary.	A axillary.	B axillary.	A axillary.	B axillary.
Plant 1.	11	0	17	0	—	—
2.	0	0	0	0	3·5	4
3.	3·5	0	5	0	6·5	0
4.	0	0	0	0	0	0
5.	0	0	6	3	6	4
6.	0	0	4	2·5	9	2·5
7.	5	3	5	4	7	6

TABLE II. *Controls.* Plants split from base of epicotyl down through main root, and decapitated in epicotyl.

September 1924. Temp. 60°–65° F. Outgrowth of axillaries in millimetres.

	6 *days.*	8 *days,*
Plant 1.	12 and 0	17 and 3
2.	7 and 5	20 and 23
3.	8 and 0	30 and 4

The fourteen control plants reported below, in Table VI, will also serve for comparison with this experiment, since they also had split roots and were growing at about the same temperatures.

From Table I it can be seen that in all plants except the first the ' A ' axillary either failed entirely to grow out for the first eight days, or was very much delayed. This must have been due to its connexion with the shoot-apex through the pith, for the controls (seventeen in all) show that the splitting of the hypocotyl and main root does not appreciably delay the outgrowth of axillaries.

It is the stronger of the two axillaries of the controls that should be taken for comparison, for their growth is not independent. The weaker one tends to be inhibited by the stronger.

At an earlier date also, with temperatures of over 70° F. (July 7, 1924), five plants were operated on as above, and compared with six controls. The controls, which had been split from base of epicotyl down through the root, and then decapitated, all showed an outgrowth of axillaries to 4 mm. or more after four days. Of the experimental plants, two showed early and strong outgrowth of the ' A ' axillaries, but in the other three the lengths of the ' A ' axillaries were, after six days, only 5 mm., 8 mm., and 4 mm., after ten days only 6, 13, and 7 mm. respectively. In these three, therefore, the ' A ' axillaries had begun to grow out (though less soon than those of the controls), but had been very noticeably delayed in further growth.

In case any new conducting tissues should have been formed, four of the plants of Table I (Nos. 2, 5, 6, and 7) were examined microscopically after 12 or 13 days. Along the longitudinal strips where the xylem of the epicotyl had been removed, the outer layers of the pith had divided tangentially to form a certain amount of large-celled parenchymatous tissue, but neither here nor at the level of the upper cut, nor anywhere in the pith, had any new conducting tissues been formed.

From the above results, it is clear that a connexion with the apex by the pith alone will allow inhibition to act to some extent, sufficiently to delay the outgrowth of an axillary very greatly, though usually not to prevent it from growing at all.

This result would be difficult to explain on any nutritive theory of inhibition. For the experiments showed that, as might be expected, the

apex cannot draw nutriment through the pith to any significant extent. For whereas the ' B ' cotyledons (connected by vascular tissue) were found to become exhausted fairly soon, the ' A ' cotyledons retained their reserves for very much longer. There must, of course, have been some demand upon them from the growing roots.

McCallum records that he made a series of cuts in a spiral up the epicotyl of *Phaseolus* so as to sever all the vascular strands : the axillaries did not grow out (9, p. 242). This result is interpreted by Fitting (5, p. 89) as showing that inhibition must be able to travel by the pith. But this it does not really show, for there would still be continuous connexion through xylem, and possibly through phloem too, by a spiral path, though not in a straight line. Indeed, the xylem connexion must still have been sufficient to supply the upper part of the shoot with water, since otherwise it would have perished.

3. *Inhibition through Xylem together with a few Cell Layers of Pith only.*

In an attempt to determine whether inhibition can act through the living xylem, the following experiment was carried out. Seedlings of *Phaseolus* were ringed in the epicotyl, and then in the ringed zone a cut was made that severed the xylem on one side (the ' A ' side) only, and penetrated the pith. Next, the epicotyl was grasped on each side of the ring, and at the same time stretched lengthwise and slightly bent with the cut side convex. In this way, the pith was made to snap apart, right across to the xylem of the other, or ' B ' side, or very nearly so. The xylem of the ' B ' side remained unbroken. All exposed surfaces were then vaselined, including those of the crack that had severed the pith. The ' A ' axillary was removed, and attention given to the ' B ' axillary (on the side with continuous xylem) alone.

Unfortunately this method does not usually strip the xylem completely. On microscopic examination, it was found afterwards that usually from two to four layers of the pith had remained unbroken and were adhering to the xylem. From these, after ten days, a considerable amount of new parenchyma had been regenerated. But for the first few days after operation the connexion must have been only by the xylem of one side and from two to four cell layers of pith, or sometimes even less.

In two plants, none of the old pith was seen unbroken, but it cannot be said for certain that there may not have been one unbroken cell layer. The results of the final series of experiments are given in the following table :

EXP. 4. TABLE III. Plants 'snapped' through the pith.

September 1924. Temp. 60°–65° F. Growth of 'B' axillaries in milli-
metres. Asterisks show date when 'B' cotyledons first became ex-
hausted.

	6 days.	8 days.	10 days.	Number of cell layers of old pith remaining unbroken, besides regenerated parenchyma.
Plant 1.	0*	0	—	2 to 3
2.	0	0	—	—
3.	0*	0	—	—
4.	0	0*	—	none seen
5.	2·5	3*	4	none seen
6.	3	4·5*	4·5	2 to 3
7.	3	4	4	2 to 3
8.	0	0	0	1
9.	7	12	20*	—
10.	0	0	—	4 to 6
11.	0	0*	0	3 to 4
12.	0	0	0	—
13.	6	10	12	2 to 3

Controls for the above experiment are given in the following table :

TABLE IV. Controls decapitated in epicotyl, with one axillary removed.

September 1924. Temp. 60°–65° F. Outgrowth of axillaries in millimetres.

	6 days.	8 days.
Plant 1.	8	18
2.	7	15
3.	7	14
4.	9	20
5.	7	—
6.	13	—
7.	7	14
8.	9	16
9.	11	15

It should be mentioned that the last six plants of these controls were
very slightly younger than those of the experiment.

From these results it is clear that a connexion by living xylem,
together with only two to four cell layers of pith, still allows inhibition to
act. For in eight of the plants the remaining axillary did not grow out ;
in three it grew out only for 4·5 mm. or less, and stopped ; in only two did
it grow out unchecked. The upper parts of the shoots continued to grow
rapidly, and gradually exhausted the cotyledons, though scarcely connected
except by the xylem. This might at first seem to favour a theory of
inhibition by nutritive exhaustion, but such an interpretation will not hold,
since, as will be shown later, it is possible to interrupt inhibition without
stopping the stream of sap in the vessels.

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But if inhibition is considered as the conduction of some influence from apex downwards, there appears no reason why the xylem parenchyma, which contains living protoplasm, should not conduct it, just as other living tissues do.

The above experiment, though simple, has to be carried out rather carefully if it is to succeed, and with plants that are not too young. Thus on an earlier occasion, when seven plants were similarly operated on at a slightly younger stage, the axill aries of all except one grew out about as rapidly as in the controls.

As to the phloem and cortex, there is no reason to doubt that inhibition can act through them also. But in *Phaseolus*, to separate them from the wood without injuring them, in order to prove the point, would be difficult.

II. Simultaneous Growth of Axillaries and Main Apex.

It was found by Newton Harvey (16) that if a zone of the epicotyl of a *Phaseolus* seedling was killed with a jet of steam, the axillaries of the cotyledons grew out, although the growth of the main apex was not hindered. Above the dead zone roots were formed.

He concludes that since the transpiration stream must still have ascended, the formation of these roots cannot have been inhibited previously by any dissolved substances passing up from the main root system. But as regards the axillary buds, experiments of this kind do not disprove the theory of inhibiting substances; for it cannot be assumed that such substances would continue to pass downwards from the apex across the affected zone. It seems of interest, however, to record the following ways in which it is possible to interrupt inhibition in a zone of the stem even without killing it.

EXP. 5. Two seedlings of *Vicia Faba* were steamed round a zone of the first internode for 20 secs. and for 30 secs. After seven days, the axillaries of the first leaves were growing out 5 and 10 mm., and after nine days 11 and 18 mm. respectively. The main shoots had continued to grow. Yet when the latter plant was examined, it was found not only that there was no visible sign of injury to the phloem, wood, and pith, but even that the inner parts of the cortex were not visibly injured.

Hence it must be concluded that steaming just for the critical period, even if it does not visibly injure the tissues of a stem, may yet inflict on them enough physiological damage or shock to prevent inhibition from acting through them any longer. When the time of steaming was only 10 secs. or 12 secs., the axillaries of *Vicia Faba* seedlings did not grow out. On the other hand, steaming for 60 secs. was found to kill a zone of one seedling right through. Yet above this zone the shoot had remained

healthy and continued to grow, while below it an axillary grew out strongly.

It is also possible to interrupt inhibition by pressure, without stopping the growth of the main shoot, as shown by the following experiment :

EXP. 6. In three seedlings of *Vicia Faba*, a zone of the first internode was surrounded by a garter of plaster of Paris enclosed in a glass tube. After three to four days the axillaries of the first leaves of all three were strongly growing out, while above the garters the main shoots continued to grow vigorously. The parts of the stem included in the garters were narrower than the parts on each side, but had not died.

Certain other experiments indicated that it is probably only when the pressure is extremely high that inhibition is interrupted.

The most elegant and instructive method, however, of interrupting inhibition without stopping the growth of the main apex is certainly by the cold-water coil of Child and Bellamy (3), referred to previously.

Such experiments, in which the main apex continues to grow, but no longer inhibits the axillaries, are clearly in conflict with a ' nutritive ' theory of inhibition (cf. ' Discussion ' below).

III. INHIBITION ACROSS A WATERY GAP.

Several kinds of excitation are now known in plants which can be conducted across a gap filled with gelatin. This suggested an attempt to determine whether inhibition can similarly pass a watery gap. The question is here much more difficult to answer, since the experiments have to last for many days. Consequently it would be useless simply to cut off the upper part of the shoot and then replace it with the cut surfaces moist and in contact, for the upper part would then perish. The experiments were accordingly arranged in two different ways, of which the first was the following :

EXP. 7. Seedlings of *Vicia Faba* were grown in pairs closely side by side. In four pairs, the adjacent sides of the stems had their external tissues removed in longitudinal strips so as to leave flat cut surfaces, usually about as deep down as the phloem, but sometimes less deep. One plant of each pair was then decapitated, and the two were bound tightly together with the cut surfaces in contact (Fig. 2). In three other pairs, the arrangement was varied by cutting clefts in the stumps of the decapitated plants, and fitting into them strong wedge-shaped pieces of tissue from their companions (Fig. 3). The cut surfaces were kept sterile, so far as practicable, and the margins of the joints were vaselined.

The result was that in all cases the first leaf axillaries of the decapi-

tated plants grew out strongly, and apparently no less rapidly than in controls. Yet when the joints were examined microscopically by sections, after from three to six days, it was found that in all the pairs except one the cut surfaces were moist and healthy. They usually adhered tightly together, especially where they passed through procambial tissue. The remains of the cut cells had disappeared, except for their walls, which had been compressed to a single line of dead cell wall and made it possible still to recognize the line of junction.

FIGS. 2 and 3.   Diagrams to illustrate Experiment 7.

Three pairs of *Phaseolus* seedlings also were similarly bound together, and in these the cotyledonary axillaries of the decapitated members of each pair grew out unchecked. Yet the cut surfaces were quite healthy, and in one pair were closely adhering, a vigorous regeneration of new tissue having set in from the cambium.

From the appearances it would certainly be expected that substances would diffuse easily across the junction, but to test whether this was so, another five pairs of *Vicia Faba* seedlings were similarly bound together, and then when the axillaries of the decapitated shoots had begun to grow out strongly, a solution of $LiNO_3$ of 1 per cent. was supplied to the cut ends of petioles of the longer shoots above the joint for twenty-four or forty-eight hours. The shorter shoots were then tested spectroscopically for lithium. In three of the pairs the lithium, though it had passed down the longer shoot, was not found in the decapitated shoot; but in the other

two pairs it had crossed the joint into the decapitated shoot, and in one of them it gave an intense colour in the actual outgrowing axillary.

Since therefore the lithium salt passed across, although inhibition did not pass across, this result tends to show that the passage of inhibition does not consist *simply* in the downward movement of an inhibiting substance; though, on the other hand, the inhibiting substance, if there were one, might for some reason not be able to follow the same path through the tissues as the lithium salt. But in any case it is still possible that inhibition may travel by some complex process into which the movement of a soluble substance enters as one stage. For if so, then inhibition may have been prevented from reaching the axillaries by the intervening length of stem of the decapitated shoot, in which the physiological conditions may have been abnormal. Accordingly the following experiment was arranged, somewhat similar to Experiment 3, above.

EXP. 8. Twelve *Phaseolus* seedlings were similarly split in their lower parts by a longitudinal split passing from the base of the epicotyl down between the cotyledons and out through the hypocotyl and main root (Fig. 4). But this time the split was also continued farther up, to a level about 2 cm. up the

FIG. 4. Diagram to illustrate Experiment 8.

epicotyl. The halves were then at once bound tightly together again, having been kept as sterile as practicable, by bandages extending from close to the upper level of the split down to just below the cotyledonary node. The margins of the split were vaselined, and one of the halves (the ' A ' half) was completely cut through above by a cut passing from the top of the split out to the side of the epicotyl. Thus as a result one of the halves (' B '), with its cotyledon, axillary, and separate root system, was still connected up with the apex of the shoot, while the other (' A ') had no longer any protoplasmic connexion with the apex, but was pressed against the ' B ' half along extensive and exactly fitting cut surfaces. The results are given in the following table :
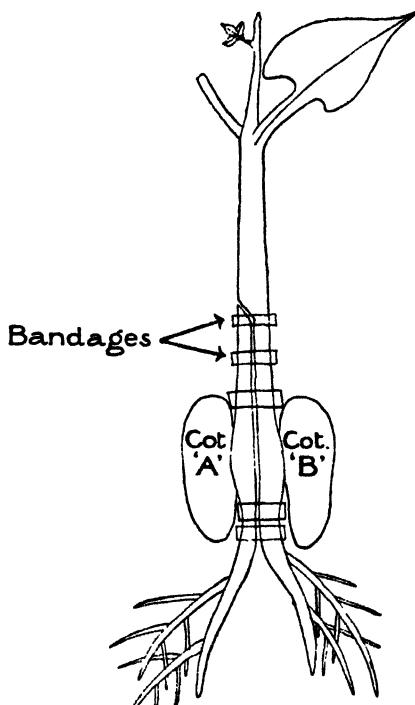
TABLE V. *September* 1924. Temp. 60°–65° F. Growth of axillaries in millimetres.

	6 days.		8 days.		10 days.	
	A axillary.	B axillary.	A axillary.	B axillary.	A axillary.	B axillary.
Plant 1.	.7	.4	11	.6	14	.10
2.	.1	2	.1	2	—	—
3.	2	0	—	—	—	—
4.	2·5	1·5	.4	3	—	—
5.	.7	2·5	13	3	—	—
6.	4·5	1·5	6	2	.8	2·5
7.	2	0	3	0	5	0
8.	.4	4	5	5	—	—
9.	.6	6	.8	6	—	—
10.	4	4	.5	5	—	—
11.	.7	0	.9	2	—	—
12.	5	0	.7	0	—	—

Controls are given in the following table. They were made on the same dates and from similar plants. All were split in halves through the hypocotyl and main root. The first six were split only so far up as just to the cotyledonary node, but the remaining eight were farther split up to 2 or 3 cm. up the epicotyl, and the last five were also tightly bound just above and below the cotyledonary node, so as to imitate the plants of Experiment 8 more closely.

TABLE VI. *Controls. September* 1924. Temp. 60°–65° F.

	6 days.	8 days.	10 days.
Plant 1.	6 and 3	10 and 5	15 and 8
2.	6 and 5	8 and 7	14 and 10
3.	8 and 7	22 and 16	—
4.	16 and 6	22 and 10	—
5.	11 and 7	20 and 11	—
6.	2·5 and 2·5	5 and 4	10 and 7
7.	9 and 5	15 and 10	22 and 12
8.	12 and 9	18 and 15	25 and 16
9.	8 and 6	10 and 8	13 and 10
10.	12 and 6	15 and 10	17 and 8
11.	10 and 8	15 and 10	28 and 20
12.	15 and 10	20 and 10	35 and 15
13.	5 and 3	8 and 5	—
14.	12 and 6	17 and 9	—

From these two tables, it can be seen that in the experimental plants the 'A' axillaries did grow out, but in most of them only very slowly. In most the 'B' axillaries also grew, though still more slowly. The reason why the 'B' axillaries should grow at all is not clear. In the controls the outgrowth was much more rapid. The mean outgrowth of the axillaries of

the controls after six days is 9·3 mm., with a standard deviation of 3·65. In the experimental plants, the mean outgrowth of the 'A' axillaries after six days is 4·3 mm., with a standard deviation of 2·04. The standard error for the difference of the means is $\sqrt{\dfrac{6_1^2}{n_1} + \dfrac{6_2^2}{n_2}} = 0·94$. The difference of the means, which is 5·15, is therefore 3·62 times its standard error, and so fully significant.

The difference between the results after eight days is much greater still, but after this period it is just possible that protoplasmic reunions may have taken place, and this question must next be considered. Of the experimental plants, Nos. 3 and 2 were examined after six days and eight days respectively. After the bandages were removed, the halves fell apart readily, showing that they were not even adhering together. On the other hand, Nos. 4 and 5, when examined after nine days, were found to have their halves adhering firmly. Under the microscope it was seen that in most parts it was the tissues outside the wood that were adhering, the halves of the pith having contracted apart. Some new tissue, consisting of radial rows of parenchyma, had been formed by divisions in the cambium and in the inner part of the cortex close to the plane of contact, and in some sections the position of the original plane of contact between the halves could no longer be recognized. It does not seem likely that after only nine days there should have been true protoplasmic reunion, by new protoplasmic filaments perforating the cell walls ; but still the possibility cannot be excluded, and therefore the results after six days are to be preferred. Even in these it would have been desirable to examine several plants, though the two examined after six and eight days were indeed selected as showing the least trace of growth of the 'A' axillary.

These results are therefore considered to show that in some circumstances inhibition can act across a moist protoplasmic gap, though only with difficulty. The further interpretation will be discussed below. The results are again in conflict with a nutritive theory of inhibition. For it is clear that the main nutritive supply by the sap stream in the vessels was interrupted, since the 'A' cotyledons were not exhausted till much later than the 'B' cotyledons.

The advantages of the arrangement of Experiment 8 are probably that like tissues are exactly fitted together, and that the watery gap is much nearer to the axillary.

DISCUSSION.

The above results show that, as already concluded by McCallum (9) and Mogk (10), it cannot be by drawing to itself the nutritive supply, and so

'starving' the axillary buds, that the main apex inhibits their growth. For on the one hand it is possible to interrupt the inhibition, and so enable the axillaries to grow out, without notably diminishing the supply of nutriment to the main apex, which goes on growing (Experiments 5 and 6, and results of Child and Bellamy); and on the other hand it is possible entirely or almost entirely to interrupt the nutritive supply from the neighbourhood of one of the cotyledons to the main apex without releasing the axillary of that cotyledon from inhibition (Experiments 3 and 8). From observations on the rate at which the cotyledons are exhausted after various operations, it seems that in *Phaseolus* seedlings the greater part of the nutriment passing up from cotyledons to main apex goes up with the stream of sap in the vessels. But this stream of sap has no inhibiting effect: inhibition passes by way of the living and physiologically active tissues.

It might be suggested, however, that the apex inhibits by drawing to itself not the general nutritive supply, but certain special substances necessary for growth. This suggestion would still be in conflict with the experiments showing that it is possible to interrupt inhibition without stopping the growth of the main apex: for so long as it is growing, the main apex must still be drawing any such substances to itself. But on the other hand, if it were supposed also that the growth-promoting substances travelled up the stem, not with the sap stream in the vessels, but in some other tissue, then the suggestion would no longer be in conflict with Experiments 3 and 8, which show that it is possible to stop the main nutritive supply by the sap stream in the vessels without interrupting inhibition.

But in return this suggestion would bring further difficulties of its own. For if the growth-promoting substances moved by diffusion, aided perhaps by random stirring and mixing movements in the cells, then their concentration would have to be *lower* at the main apex than at the axillary buds which are supposed to be inhibited for lack of them. And this difference in concentration would sometimes have to be quite large, in order to maintain a sufficient gradient for diffusion. For McCallum has shown that in *Phaseolus* inhibition can extend over nine internodes.

It may be suggested, however, that the growth-promoting substances are translocated by a definite mechanism, which normally works towards the main apex, but, after removal of the apex, changes its direction, and moves the substances into the axillary buds. Since, however, it is removal of the apex which is assumed to cause this change in direction of translocation, it follows that, when present, the apex must have been determining the direction of translocation towards itself. That is to say that it must have been transmitting back some kind of influence down the shoot. But this is exactly the conclusion which is here advocated, and which it is the object of a 'nutritive' theory to avoid. This last suggestion, therefore,

does not avoid the conclusion that some influence is transmitted down the shoot from the apex, and further meets with the difficulty that the assumed translocating mechanism must be supposed to be present in the relatively unspecialized pith, amongst other tissues: for the pith can transmit inhibition (Experiment 3).

There is therefore no escape from admitting that inhibition is brought about by some influence that originates at the growing apex, is conducted thence to other parts of the plant, and directly or indirectly prevents the axillary buds from growing out. This conclusion agrees with the views of Jost (7) and Fitting (5).

It must, therefore, next be considered in what way inhibition is conducted down the shoot from the apex. It has been shown that in *Phaseolus* inhibition can be conducted across a watery gap. It is extremely probable that the various kinds of excitation in plants which can be conducted across a watery gap or a gap filled with gelatin (1, 12, 13, 14, 15) are conducted across by the diffusion of soluble stimulating substances. This view is strongly supported by experiments by Stark (14, p. 110, and 15). In a general way, also, if stimulating substances are involved, it is easier to see how various different excitations or correlative influences can be conducted through a tissue at the same time : for they may simply depend on different substances. It therefore seems probable that inhibition also is conducted across the gap by the movement of a soluble substance.

It is, however, also possible, though less likely, that it is conducted across by a local electric current. Or again, if the watery gap becomes permeated with substances diffusing out of the tissues, then subsequently conduction across it may possibly be a change propagated by means of the molecules of the dissolved substances, but yet not consisting simply in their diffusion across the gap.

But even if in the watery gap excitation or inhibition is conducted by the diffusion of a soluble substance, as seems most likely, it does not at all follow that in the tissues it is conducted simply by the movement of such a substance all the way. For the conduction of excitation may be a complex process, as suggested by Páal (11, p. 432), in which excitation at one point in a tissue sets free a stimulant which, moving to neighbouring points in the tissue, excites them in turn to produce more stimulant, and so on. Similarly it seems on the whole more probable, partly on account of the result of Experiment 7, and partly for other reasons, that the conduction of inhibition is some such complex process, rather than that it consists simply in the movement of a soluble inhibitor all the way. But the conclusion is not certain, and, since it is intended to experiment further, it will not be further discussed at present.

SUMMARY.

1. It is pointed out that the axillary buds of a leguminous seedling must somehow, directly or indirectly, be prevented from growing out by the apex of the main shoot. This influence exerted upon them by the main apex, whatever its nature may be, will be called ' inhibition '.

2. In *Phaseolus* inhibition is not interrupted by ringing the epicotyl down to the wood, even for a length of 6 to 8 cm.

3. Inhibition is not completely interrupted, though it is weakened, when an axillary is left connected with the main apex only by the pith.

4. Inhibition is not interrupted when axillary bud and main apex are connected only by the living xylem, with one or two cell layers of pith parenchyma adhering to it.

5. When a zone of the stem of *Vicia Faba* is subjected to a jet of steam for 20 or 30 seconds, inhibition is interrupted by the physiological shock, although it is only the outer parts of the cortex that are killed. The axillaries grow out below the zone, while the main apex still goes on growing above it.

6. The same result can be produced by strongly compressing a zone of the stem in a garter of plaster of Paris.

7. When the stems of seedlings were bound together in pairs, with cut surfaces of tissue pressed together, and one of each pair was decapitated, inhibition was not able to pass across from one to the other. Yet lithium nitrate was found sometimes to pass across.

8. With a more favourable arrangement, however, by which the lower parts of single *Phaseolus* seedlings were cut right through longitudinally in a certain way and then bound up again, inhibition did act across a watery gap.

9. It is concluded that inhibition of the axillaries cannot be brought about simply by nutritive exhaustion, but must depend on some conducted influence that originates at the growing apex.

10. It is also concluded that this conduction of inhibition is probably a process into which the movement of a soluble substance enters, at least as one stage. But it does not at all follow that the conduction consists simply in the movement of such a substance all the way.

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Mercurialis.

III. A Consideration of the Physiological Significance of the Chromogen.

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I N a recent publication [1] attention was drawn to the occurrence in *Mercurialis perennis* and *M. annua* of a labile chromogen which could be anaerobically extracted from the plant. On exposure to air the colourless solution absorbs oxygen and turns blue, and this blue, on further exposure to air, changes through green to yellow, the depth of which depends upon the strength of the solution.

Although we have not as yet been able to determine the chemical constitution of these various compounds, we have thought it desirable for the sake of precision to describe the chromogen by the name Hermidin [2] and the blue and yellow derivatives as cyano- and chryso-hermidin respectively.

It is the interrelationships of these three substances which form the subject of the present communication, in view of their possible significance in the respiratory mechanism of the plant.

Owing to the fact that the oxidation of the chromogen takes place in two distinct phases, as is indicated by the marked colour changes, it was thought to be of interest to ascertain the volumes of oxygen involved in the two stages of oxidation. To determine this, a given volume of chromogen solution was shaken up with excess of air in a eudiometer, the contraction in volume being noted at the moment when the blue colour due to cyano-hermidin had reached its maximum ; the oxidation to chrysohermidin was

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[1] Haas and Hill: Biochem. Journ., 1925, xix, pp. 233, 236.

[2] The term Hermidin (derived from Hermes) has been chosen to indicate the connexion of this substance with the parent plant, the more obvious term Mercurialin having been applied many years ago to the base methylamine at the time of its isolation from *Mercurialis perennis*.

then completed and the total volume of oxygen absorbed was noted. These experiments were carried out on extracts from material gathered at different times and from various localities. The results are set forth in the following table, together with the quotient of the volumes of oxygen absorbed :

TABLE I. *Amount of Oxygen in c.c. absorbed by 20 c.c. of Extract.*[1]

Chromogen to Blue.	Complete Oxidation.	Quotient.
2	3·7	0·54
1·9	3·2	0·59
2·7	4·0	0·66
1·9	3·8	0·47
1·6	2·6	0·61
1·8	3·3	0·62
1·3	2·9	0·54
1·6	3·0	0·54
1·7	3·6	0·47
1·1	2·2	0·50

From the average value of 0·55 given by the figures in the last column, it appears that the volume of oxygen absorbed in the first stage is half the total volume—that is, the volumes absorbed in the two stages are equal.

It may be argued that the figures in the third column of the table are too divergent to justify so definite a conclusion, but it must be pointed out that there are two sources of error. Firstly, during the transfer of the chromogen solution from the generating vessel to the eudiometer a certain amount of oxygen is unavoidably absorbed, and this quantity is not measured. Secondly, it is difficult to determine the precise moment of attaining the maximum depth of blue ; the end-point, therefore, must be judged by the first appearance of green in the solution. There is thus a tendency to over-estimate oxygen absorbed in the first phase of the oxidation of the chromogen, and this error tends to make the quotient too high.

The oxidation takes place with great rapidity, the strongest extracts obtained being completely oxidized when vigorously shaken for less than a minute. In view of this rapidity and the considerable volumes of oxygen involved, it was thought desirable to ascertain whether oxidizing enzymes are able to bring about the absorption of similar quantities of oxygen at comparable rates. The potato tuber and horse-radish root were selected for the purpose. They were anaerobically extracted and subsequently examined under precisely similar conditions to *Mercurialis* : whilst the extract of *Mercurialis* absorbed 4 c.c. of oxygen, no measurable absorption of this gas was effected by the extracts from the other two plants.

The avidity for oxygen manifested by hermidin may be better realized when it is remembered that 1 grm. of haemoglobin absorbs 1·3 c.c. of

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[1] Made from cut-up green shoots covered with about five times their weight of water.

oxygen; in the case of *Mercurialis* it was estimated on one occasion that the extract from 4 grm. of the cut-up plant, representing less than 1 grm. of dry weight, and containing but a small proportion of chromogen, absorbed 4 c.c. of oxygen.

The question of reversibility may next be considered. It has already been shown [1] that the oxidation of the chromogen to cyanohermidin is reversible. The addition of a small quantity of sodium hydrosulphite will decolorize the blue solution ; on shaking with air the blue colour may be restored. The reduction of the fully oxidized yellow chrysohermidin is not effected by the same reagent, but may be brought about by the use of an aluminium mercury couple which also may be used for the reduction of the blue stage to chromogen. These are, however, but test-tube experiments ; the degree to which the plant is able to bring about a similar reduction is all-important.

In the process of extraction, although oxygen is eliminated as far as is possible from the vessel, there is always some residual oxygen left in the air-space system of the plant, but in spite of this fact the final extract of fresh material is not blue, so long as atmospheric oxygen is excluded. In the course of extraction it has, however, often been noticed that a blue colour may be developed and later disappear, and it was thought that this disappearance was due to the further oxidation of the cyanohermidin by the residual oxygen. The observations on the reduction of the cyanohermidin in the test-tube led to attempts to effect the same reduction by means of the plant. Extracts were accordingly made in the ordinary way, filtered and shaken up with air until the desired depth of blue had been attained. A number of fresh shoots were then submerged in the blue solution contained in a wide-mouthed bottle, the stopper was replaced, and a current of nitrogen bubbled through the solution for a length of time sufficient to wash out the oxygen as far as possible. The inlet and outlet were then closed and the bottle sunk in a vessel of water in order to keep it air-tight. In about twelve hours the solution was decolorized. On shaking up this solution with air, the blue colour reappeared and could once more be made to disappear by the same shoots, but such reduction could not be repeated indefinitely. This observation has many times been made ; the experiment is most effective when young white rhizomes are used for the source of chromogen, the complication due to chlorophyll is eliminated, a cleaner extract is obtained, and a better judgement of the depth of colour is possible.

In carrying out such a reduction experiment, it is essential to guard against the addition of too much tissue, for there is a danger of destroying the blue colour by oxidation to chrysohermidin rather than by reduction to chromogen, owing to the amount of air introduced in the intercellular air

[1] Haas and Hill : loc. cit.

spaces. If, however, a relatively small amount of tissue is added, the reducing action predominates over any oxidation which may be set up by the intercellular oxygen, and the result is that the blue is destroyed by reduction and not by oxidation. This is indicated by the fact that on shaking up with air the blue is once more produced ; had the destruction of the blue been due to oxidation to chrysohermidin, further shaking with air would have produced no effect.

Attempts to bring about the reduction of the chrysohermidin to cyano-hermidin by means of green tissue have not met with much success, but it has been noticed more than once in gas absorption experiments that the yellow solution, on standing overnight in the eudiometer, sometimes is of a bronze colour next day, with a zone of pure yellow at the top. This suggests that a certain amount of reduction to cyanohermidin may have taken place during the night, and this, mixed with the yellow chryso-hermidin, may have given the bronze colour. The yellow zone at the surface in contact with the air contained in the eudiometer is produced by re-oxidation of the cyanohermidin. In one such experiment it was found that, on shaking up again, a further absorption of o·4 c.c. of oxygen resulted and the bronze colour gave way to the yellow of fully oxidized chryso-hermidin.

In drawing conclusions from the observations made, caution is necessary : it is, however, considered that the balance of evidence indicates that there exists in the plant an agent capable of reducing the cyano-hermidin to the colourless chromogen. Whether the plant is also capable of reducing chrysohermidin to cyanohermidin is not so evident. This, perhaps, is not surprising in view of the fact that in the test-tube, sodium hydrosulphite, although able to effect the reduction of the blue compound, cannot reduce the yellow.

In attempting to evaluate the physiological significance of hermidin, it must be remarked that this substance is most abundant at those periods where development and respiration are most intense.[1] It is not, however, suggested that this incidence is proof of the participation of the chromogen in the respiratory process : the chromogen may itself be a by-product of respiration or of some other metabolic activity. On the other hand, if the chromogen really does play a part in the respiratory process, then it should be most abundant at those phases in the life-history of the plant during which respiration is known to be most intense.

It has been shown that hermidin exhibits a marked avidity for oxygen' yielding cyanohermidin and chrysohermidin, and that both of these oxida-tions may be reversed by suitable chemical agents. Cyanohermidin may

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[1] For example, it was found that equivalent weights of young aerial shoots gathered on 22nd April and the tips of mature aerial shoots collected on 8th June, from the same locality, absorbed 4 c.c. and o·54 c.c. of oxygen respectively.

be reduced by the plant, but the nature of the reducing agent is not as yet known. Evidence concerning the reduction of chrysohermidin by the plant is less easy to obtain, but its reduction by the living plant would appear to be of little or no importance, since under normal conditions cyanohermidin would, presumably, not be formed.

Although an absolute proof cannot be given, the balance of evidence supports the view that hermidin plays a part in the respiration of the plant, more particularly in the absorption of oxygen. The working hypothesis suggested is that the cyanohermidin so produced is immediately reduced, losing its oxygen to the reducing agent, which may or may not be a metabolite.

To what extent this mechanism obtains in other plants is a matter for investigation; we have not, as yet, found[1] any plant which shows a similar phenomenon, though we hope in due season to be able to examine *Boletus cyanescens* from this point of view.

Hermidin itself may prove not to have a wide distribution; but comparable substances, which do not give a coloured compound on oxidation, may occur, although their existence has not hitherto been suspected. Their presence can only be indicated by their oxygen-absorbing properties. Even supposing that no compound analogous to hermidin exists in other plants, this fact alone should not militate against the respiratory significance of hermidin in the few examples in which it does obtain. The plant exhibits its plasticity in no small degree in the many and diverse methods it employs for obtaining internal energy.

SUMMARY.

1. From *Mercurialis* may be extracted a colourless chromogen, hermidin, which shows a marked avidity for gaseous oxygen.

2. Hermidin undergoes oxidation in two stages, yielding a blue compound, cyanohermidin, and a yellow compound, chrysohermidin. The volumes of oxygen fixed during these two stages are equal.

3. By suitable chemical means cyanohermidin may be reduced to hermidin and chrysohermidin to cyanohermidin.

4. There exists in *Mercurialis* a mechanism capable of reducing cyanohermidin to hermidin.

5. It is suggested that hermidin plays a part in the respiratory mechanism of the plant, more especially in the absorption and transfer of oxygen.

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[1] We are indebted to Dr. A. B. Rendle, F.R.S., for drawing our attention to *Argyrothamnia* and *Claoxylon*, two genera of the Euphorbiaceae, as possibly exhibiting similar phenomena, but we have unfortunately not been able to obtain fresh material of these plants.

On the Development of Buds upon Roots and Leaves.

BY

THEO. HOLM.

AS a means of vegetative reproduction, the development of buds upon roots and leaves plays a rôle of no small importance. In some cases there is a very distinct correlation between the development of root-shoots and the structure of the rhizome, or even the production of germinative seeds. Shoots of that kind are known from Selaginellaceae, Ophioglossaceae, Polypodiaceae, and from many Angiospermae, but not from the Gymnospermae. Most of the plants in which root-shoots occur are trees and shrubs; they are relatively rare in herbs, and confined almost exclusively to the perennials. The only cases known from annuals are, according to Karsten, the development of an inflorescence from the apex of an aerial secondary root in the cultivated *Impatiens Balsamina*, L.; furthermore Wittrock found root-shoots in the biennial *Erythraea linariaefolia* (Lam.), but only in about a dozen of several thousand specimens examined; finally, the biennial *Gnaphalium arenarium*, L., *Alliaria officinalis*, Andrz., and *Brassica Napus*, L., exhibit this same peculiarity according to the writings of Alexander Braun, Caspary, and Wydler. But otherwise the herbs from which root-shoots are known are all perennials.

The subject has never attracted much attention, still it was known, some 350 years ago, to Clusius (*Convolvulus althaeoides*, L.) and Dodonaeus (*Lepidium latifolium*, L.); the internal structure was treated as early as 1847 by Trécul, who examined *Paulownia*, *Tecoma*, *Ailanthus*, and *Maclura*. Since then we have several morphological studies presented notably by Alexander Braun, Caspary, Irmisch, Warming, and Wydler, who included also the development of buds upon the hypocotyl. Finally, Warming in 1877-9 and Wittrock in 1883 have given us very complete lists of herbs developing root-shoots, comprising 124 Dicotyledons, eight Monocotyledons, and six vascular Cryptogams (Wittrock).[1]

[1] Balanophoraceae and Rafflesiaceae are not included.

In considering the 'root-shoot', we find this comprises actually two distinct types with the shoot terminal or lateral. In the former case the apex of the root becomes transformed into a shoot, as is the case of some ferns: *Diplazium (Asplenium) esculentum*, and several species of *Platycerium* (Goebel); furthermore some Monocotyledons, viz. *Listera* (Brundin), *Neottia* (Vaucher), *Pogonia* (the author), and *Anthurium* (Goebel). But in *Ophioglossum vulgatum* the shoot is not terminal; it becomes developed very close to the apical cell of the root, from one of its youngest segments, while the apex of the root continues its growth undisturbed (Rostowzew). Finally, in the Selaginellaceae Pfeffer observed that the so-called rhizophores may become transformed into terminal leafy shoots.

But regarding the Dicotyledons terminal root-shoots are evidently very rare, and the literature does not give us any precise idea about the actual occurrence of such shoots.

With special reference to the herbs, Wittrock (loc. cit.) has classified the root-shoots in accordance with the conditions under which they occur and their importance to the vegetative reproduction: 'reparative' being such which develop only in cases where the root becomes injured or torn off from the mother-plant; 'additional', which develop spontaneously upon roots of uninjured specimens, hence forming a more or less important addition to the reproductive power of the individual; finally, 'necessary' are such as constitute a part of the normal morphological development of the plant.

In passing, to describe some types of these shoots, the trees and shrubs will be included.

I. REPARATIVE ROOT-SHOOTS.

The material studied by Trécul consisted exclusively of pieces cut from roots of *Ailanthus*, *Paulownia*, *Tecoma radicans*, and *Maclura aurantiaca*. While in *Paulownia* and *Tecoma* the shoots developed from any part of the root except at the surface of the wound, this author observed in *Ailanthus* that shoots developed from the uninjured sides of the root as well as from the surface of the wound; in *Maclura*, on the other hand, the surface of the wound was the only place where they appeared. In comparing these observations with the natural habit of these plants, it is a fact well known that they multiply naturally in this manner without having been exposed to injury. In other words, Trécul's observations demonstrate that roots cut into fragments become stimulated, so as to produce shoots from the uninjured lateral part or from the surface of the wound. Reparative and additional shoots may thus occur on the same plant, trees, and shrubs in this particular case. Similar cases are also reported by Wittrock from certain herbs.

But with respect to trees and shrubs reparative shoots may be a common occurrence, although the additional type is undoubtedly the natural one; we need only to mention *Sassafras, Robinia, Aralia spinosa,* all the species of *Rhus,* &c. On the other hand, the few herbs, *Bunias, Trichera, Crambe,* and *Corydalis,* may only exhibit this peculiarity when injured.

II. ADDITIONAL ROOT-SHOOTS.

This type occurs spontaneously upon uninjured roots, and the majority of plants known to produce root-shoots represent this type. It is exemplified by many trees and shrubs, and among North American representatives may be enumerated: *Amelanchier, Aralia spinosa, Azalea, Calycanthus, Castanea. Clethra, Comptonia, Diervilla, Diospyros, Gymnocladus. Hydrangea, Myrica, Populus, Prunus, Rhus, Robinia, Rubus, Salix, Sapindus, Sassafras, Staphylea, Viburnum, Tecoma, Xanthoxylum.*

In tropical America *Artocarpus incisa,* L., depends solely upon multiplication by means of root-shoots, which occur spontaneously and in great abundance. As stated by de Candolle, the cultivated varieties of this tree develop no seeds, and their wide distribution in the tropics depends upon the facility with which they are propagated by cuttings and root-shoots.

With reference to the herbs, this method of multiplying is, as stated above, known from a relatively small number of species. In the following enumeration we have only included the species which we have found on this continent, and which are not included in the lists of Warming and Wittrock.

Orchidaceae: *Habenaria repens,* Nutt.; *Pogonia ophioglossoides,* (L.) Ker; *Isotria verticillata,* Raf.
Ranunculaceae: *Anemone canadensis,* L.; *Hydrastis canadensis,* L.
Cruciferae: *Parrya macrocarpa,* R. Br.
Leguminosae: *Astragalus aboriginorum,* Rich.; *Lupinus perennis,* L.; *Strophostyles umbellata,* (Muehl.) Britton; *Lespedeza virginica,* (L.) Britton; *L. capitata,* Michx.; *L. violacea,* (L.) Pursh; *Stylosanthes elatior,* Sw.; *Tephrosia virginiana,* (L.) Pers.; *Desmodium marilandicum.* (L.) DC.; *D. rigidum,* (Ell.) DC.; *D. obtusum,* (Muehl.) DC.
Cistaceae: *Helianthemum canadense,* (L.) Michx.
Violaceae: *Viola pedata,* L.
Pyrolaceae: *Pyrola aphylla,* Sm.; *P. picta,* Sm.; *Chimaphila maculata,* (L.) Pursh; *Ch. umbellata,* (L.) Nutt.
Monotropaceae: *Monotropa uniflora,* L.; *M. lanuginosa,* Michx.
Apocynaceae: *Apocynum medium,* Greene; *A. cannabinum,* L.
Convolvulaceae: *Evolvulus argenteus,* Pursh; *E. sericeus,* Sw.; *Convolvulus spithamaeus,* L.; *Ipomaea leptophylla,* Torr.; *I. Batatas,* Lam.; *I. pandurata,* (L.) Mey.

Solanaceae : *Physalis pubescens*, L. ; *Ph. viscosa*, L.
Acanthaceae : *Ruellia ciliosa*, Pursh.
Compositae : *Artemisia trifida*, Nutt. ; *Iva axillaris*, Pursh ; *Thelesperma subnudum*, Gray.

In these plants the shoots had developed upon the roots themselves, while Wittrock included also such cases where the shoots were borne upon the hypocotyl. Among the species recorded by Wittrock the following were also observed on this continent as developing true root-shoots : *Rumex Acetosella*, L., *Asclepias syriaca*, L., *Pyrola chlorantha*, Sw., *P. secunda*, L., *Chamaenerium angustifolium*, (L.) Spach, *Chondrilla juncea*, L., and *Listera cordata*, R. Br.

III. NECESSARY ROOT-SHOOTS.

Wittrock enumerated *Pyrola uniflora*, L., *Monotropa Hypopitys*, L., *Thladiantha dubia*, Bunge, and *Ophioglossum pedunculosum*, Desv., as representing this type of shoots. The necessity, however, is limited to the power of rendering the individual capable of persisting from year to year, since no perennial ' normal' buds are developed. They are not, on the other hand, necessary to the maintenance of the species, since the primary shoot, developed from the seed or the spore, is fertile.

In some other plants the root-shoots are absolutely necessary, because the primary shoot dies down to the ground in the first year without having reached the flowering stage and without having developed any perennial axillary shoots. Among these are, according to Wittrock, *Cirsium arvense*, L., several species of *Linaria*, *Convolvulus arvensis*, L., *Thesium montanum*, Ehr., *Coronilla varia*, L., and some species of *Euphorbia*. To these may be added *Rhexia virginica*, L., and *Rh. mariana*, L., of the Melastomaceae.

GENERAL REMARKS ON ROOT-SHOOTS.

In comparing the lists by Warming and Wittrock with our enumeration given above, it seems as if the development of root-shoots is a rather scattered occurrence within some forty families of herbaceous plants. Moreover it is a relatively very small number of genera or species where these shoots are developed in abundance, and as a constant occurrence ; for instance, *Pogonia*, *Isotria*, *Chamaenerium*, *Helianthemum*, *Strophostyles*, *Rumex Acetosella*, Convolvulaceae, *Artemisia*, *Apocynum*, *Physalis*, Pyrolaceae.

No correlation seems to exist between the development of these shoots and environment, for even if the majority of these plants are decidedly heliophilous, and frequently inhabitants of dry and hard soil, we meet also with some sciaphilous species exhibiting this peculiarity ; for instance,

Pyrolaceae, Monotropaceae, and Orchidaceae. Among the aquatics *Sium latifolium* is the only herb observed so far (Warming). Some peculiar adaptation, however, is exhibited by some of the heliophilous species, which is readily noticeable in the first vegetation that appears in cleared woodland ; we think especially of the fire-weed (*Chamaenerium*), *Convolvulus spithamaeus*, and *Ipomaea pandurata*; conversely *Helianthemum* adopts this habit when overtaken by the woods.

Irmisch was the first author to point out the biologic fact that, as a rule, root-shoots and stolons substitute each other. Nevertheless some few exceptions from this rule are known ; for instance, the stoloniferous *Lupinus perennis*, *Ajuga genevensis*, the Pyrolaceae, and *Silene nutans*.

In some other instances the occurrence of root-shoots is characteristic of certain plants in which the seeds frequently fail to develop. Wittrock has thus called attention to the abundant development of root-shoots in *Lepidium latifolium*, L., and *Nasturtium Armoracia*, which seldom produce any germinative seeds. We have observed the same to be the case of *Convolvulus spithamacus*, *Apocynum*, and *Asclepias*, where only a few fruits are developed and a minimum of germinative seeds.

While the actual development of buds upon roots is very uniform, there is one case which, so far, appears unique. In *Scilla Hughii*, grown in the botanical garden at Copenhagen, Denmark, the roots showed numerous bulblets with fleshy, scale-like leaves, and with a young root-system of their own ; thus they were capable of producing new individuals (Warming).

Considered from a geographic point of view, the formation of root-shoots appears to be more common in the temperate than in the tropical and arctic zones. From the tropics we know of *Artocarpus*, *Evolvulus*, *Anthurium*, *Diplazium*, *Platycerium*, and *Selaginella*; from the arctic. *Parrya macrocarpa* (Herschel Island) and *Astragalus aboriginorum* (Bernard Harbour).

The earliest record of the common type of root-shoots dates back to Dodonaeus (1583), who observed them in *Lepidium latifolium*, L. In the diagnosis of this plant, which was called *Lepidium Plinianum*, Dodonaeus writes : ' Radix albida, serpit et non nullibi progerminans stirpem multiplicat velut *Rhaphanidis magnae*' (*Nasturtium Armoracia*). Wittrock has confirmed the correctness of this statement by cultivating some specimens of said *Lepidium*, and he gives an interesting account of the growth of the root-system. Some of the lateral roots, developed from the primary, grow in a horizontal direction close to the surface of the soil, about two inches beneath this ; having attained the length of about half a foot the root bends downwards very suddenly, and continues to grow in this new direction. At the knee thus formed the development of root-shoots begins, and Wittrock observed as many as six from a single root. The development of these shoots enables the root to increase in length and thickness. From this

same root, a little below the shoots, some new (two to six) horizontal roots become developed, which repeat the manner of growth of the mother-root, and produce a new set of shoots.

This very regular development of root-shoots, and of several at the same time, is evidently a rare occurrence. In *Rumex Acetosella*, L., many shoots are developed from the same specimen in a single season, but only one from each part of the root. Alexander Braun and Irmisch were the first authors to mention and describe these, in the year 1850. According to Irmisch this species of *Rumex* differs from the others by lacking a strong and thick primary root. The very slender roots, however, ramify freely and spread in all directions. While the roots are still young some small swellings appear here and there. These swellings are buds, and each of these develops into a short axis, from the apex of which a rosette of leaves becomes developed, subtending axillary shoots. The cluster of shoots, so readily to be observed in this species, is thus actually of second order, having originated from one single axis, from one single bud. In *Anemone canadensis*, L., *Helianthemum*, *Strophostyles*, *Apocynum*, *Convolvulus spithamaeus*, L., *Physalis*, *Artemisia*, and *Thelesperma* we observed the long horizontal roots bearing quite a number of shoots, but all single and at some distance from each other. We counted as many as ten large flowering stems developed from a very thick horizontal root of *Apocynum cannabinum*. L., the length of which was only about one metre. *Physalis* and *Convolvulus spithamaeus* are also very productive in this respect, and the roots attain a great length. Regarding *Chamaenerium angustifolium*, (L.) Spach, Irmisch (1857) made the interesting observation that the primary as well as the secondary roots of the young seedling develop buds freely, which sometimes give rise to new plants in the succeeding year. The shoots developed from old roots may grow so fast that they bloom within a month. The roots are undoubtedly capable of persisting for several years in a dormant condition until the environment changes by the clearing or burning of the woods.

The same is the case of *Convolvulus spithamaeus*, according to our observations in Brookland, D.C., where several acres of woodland (*Pinus Virginina*) were cleared some years ago, and the scant original vegetation, mostly of *Chimaphila* and *Eupatorium ageratoides*, disappeared at once. But to our surprise numerous large patches of *Convolvulus spithamaeus* appeared, and, in spite of careful research, we found only plants developed from horizontally creeping old roots. We might mention at the same time that none of the flowers developed mature fruits, and that there was seldom more than one single flower upon each stem. Since then we have observed this same species growing under similar conditions and constantly without fruits.

These instances of roots remaining dormant for several years, and

producing shoots when the conditions become favourable, thus show the importance of this method of reproduction, and especially in cases where the species are not provided with rhizomes in the stricter sense of the word.

The genus *Apocynum*, as represented on this continent, contains several species which multiply by means of root-shoots; the popular name of *Apocynum cannabinum*, Wandering Milkweed, is undoubtedly derived from its method of vegetative reproduction. The shoots develop from the long, thick, horizontally creeping roots; they are erect, and bear several opposite scale-like leaves, some of which subtend buds that winter over and grow out as aerial shoots in the following season; the basal internodes of the shoot persist, forming a pseudo-rhizome. Although *A. cannabinum* and *A. medium* are frequent in this vicinity, and especially the former occurring in great abundance, we have never been able to detect any seedlings; all the plants which we have examined were simply root-shoots. In this connexion may be mentioned that *Apocynum*, similar to *Asclepias*, *Accrates*, and *Gonolobus*, develops only a very few fertile flowers, usually only one or two, while the inflorescence is a rich-flowered cyme. The follicles contain numerous seeds with a long coma at the summit, but we have never so far succeeded in raising any seedlings.

SOME CHARACTERISTIC TYPES OF ROOT-SHOOTS.

Orchidaceae. Terminal root-shoots have been found in some species of this family. The very first discovery of this interesting structure was made by Vaucher (1841), who observed it in *Neottia*. Some few years afterwards Reichenbach, Prillieux, and Irmisch made the same observation; besides that, Irmisch called special attention to the fact that these root-shoots did not break out from the roots, but the root appeared to change itself into a short axis, upon the apex of which the first leaf became developed.

In *Listera cordata* described by Brundin, and in *Pogonia ophioglossoides* by the author, the long horizontal roots frequently terminate in a shoot, and the first secondary root, developed from this shoot, grows out in a straight line in exactly the same direction as the shoot-bearing root, and continues the development of shoots. In *Isotria*, on the other hand, the root-shoots are lateral, and as many as twenty flowering stems may be found upon the same root. We have also observed root-shoots in *Habenaria repens*, Nutt., but the specimens were not well preserved, thus we were unable to define their exact position, terminal or lateral.

Papilionaceae. According to Wittrock's list only *Medicago lupulina*, L., and *Trifolium repens*, L., have been observed to develop root-shoots. In the North American *Astragalus aboriginorum*, Richards, collected by the Canadian Arctic Expedition (1913–18), several specimens showed root-

shoots, and these, as many as ten, were developed upon the strong primary root. *Lupinus perennis*, L., is not infrequent near Clinton, Maryland, and it may sometimes occur in great abundance owing to its widely creeping stolons and development of shoots upon the long horizontal roots. The species of *Lespedeza* and *Desmodium* enumerated in our list are perennial, and the roots are very long, frequently horizontal, and produce shoots abundantly in *L. virginiana*. *Strophostyles umbellata*, (Muehl.) Britton, which is very common in our vicinity, develops many seeds and, at the same time, many root-shoots. It is described in Gray's ' Manual ' as possessing ' a perennial root-stock ', but this statement is not correct, only the roots may persist for some years. In the course of the first season the primary stem grows out into a long purely vegetative shoot, while flower-bearing branches develop from the opposite green leaves ; the buds in the axils of the cotyledons stay dormant until the next year. The root increases slightly in length, but no root-shoots appear to be developed during the first season. Towards the end of the first season the aerial shoot dies down to the ground, leaving the hypocotyl and the cotyledonary buds beside the root, still active, to hibernate. In the second year two erect branches become developed from the cotyledonary buds, and the hypocotyl appears now as a swollen short axis ; the primary root has increased considerably in length, and bears several lateral horizontal branches. The first leaves of the cotyledonary shoots are opposite like those of the primary axis. The development of the aerial shoots does not extend any farther, and at the end of the second year the primary root is the only persisting organ of the plant. Already in the second season, however, numerous small buds become developed upon several of the roots, the primary as well as the lateral ; they persist through the winter, and in the succeeding spring they grow out into erect shoots. The vegetation of *Strophostyles* is thus exemplified by several stages of very distinct structure : the seedling in the first year, with its basal, opposite floral branches. and terminal, purely vegetative shoot ; the seedling in its second year, with both the terminal and lateral branches bearing flowers, and with the development of buds upon the roots ; the persisting primary and lateral roots in the third year, developing numerous shoots, all with the leaves alternate, and with the terminal as well as the lateral branches flower-bearing. The root-shoots themselves are also distinct, depending on their position ; those developed upon the vertical primary root are as a rule much stronger, taller, and more robust than those appearing upon the horizontal lateral roots.

It is interesting to notice that *Strophostyles* and *Melilotus* (Irmisch) show exactly the same vegetative structure during the first and second year ; but while the entire plant of *Melilotus* dies off at the end of the second season, the primary root in *Strophostyles* persists and remains active for some years.

Pyrolaceae. The necessary type of root-shoots is represented by *Pyrola uniflora*, L., the additional by the other species. Irmisch made the interesting observation that in *Pyrola secunda* the shoots were developed most frequently upon roots that had become separated from the mother-plant. In *Pyrola chlorantha* this author observed stolons, which sometimes became ramified, and, nevertheless, the roots showed frequently adventitious shoots. Concerning *Pyrola aphylla*, Sm., we have described this species several years ago (1898) and called attention to the unfortunate name ' *aphylla* ', besides the incorrect description ' species absentia foliorum spectabilis et quasi ad *Monotropeas* vergens' (de Candolle), and 'folia omnino nulla' (W. J. Hooker); as late as 1912 Warming considered the species to be ' on the way to become a holosaprophyte. possessing only small green, scale-like leaves'. Even Gray went so far as to describe it as ' leafless, doubtless parasitic '. Nevertheless, as early as 1843, Nuttall described it correctly, stating : ' Occasionally it produces, near the root and on infertile shoots, a few small, ovate. or lanceolate greenish leaves.' With reference to the other North American species, we observed root-shoots in *Pyrola picta*, Sm., *Chimaphila maculata*, (L.) Pursh, and *Ch. umbellata*, (L.) Nutt. These species of *Chimaphila*, notably *Ch. umbellata*, are stoloniferous. the stolons attaining the length of two-thirds of a metre.

Monotropaceae. Schacht has described *Monotropa Hypopitys*, L., and given an excellent figure of the plant, showing the root-system with several shoots, as well as the development of buds in the axils of the stem-leaves. *Monotropa lanuginosa*, Michx., grows in the same manner. In *Monotropa uniflora*, L., on the other hand, the roots are very short, profusely branched, and form a roundish ball, from which the flowering stems emerge during the early part of the summer. When the fruit has matured the stems die off completely, while the roots persist, and the hibernating stage may be observed already in the month of September.

Melastomaceae. Necessary root-shoots are characteristic of *Rhexia virginica*, L., and *Rh. mariana*, L. In the seedling of the former, collected in the month of August, the cotyledons are very small, epigeic, and the erect primary shoot bears several pairs of opposite leaves ; the primary root is slender, but two of the lateral roots show a distinct swelling terminated by a capillary apex with a few branches. At the end of the first season the shoot dies down to the ground, and the root-system withers with the exception of the two lateral swollen branches, which winter over as two separate roots. In the succeeding spring a young shoot develops from the small tuberous root; the first five pairs of leaves are scale-like, and secondary roots develop in pairs from the nodes between the scale-like leaves. These roots increase in length, branch freely, and are able to produce shoots in the same manner as described above. Although the subterranean portion of the stem bears several leaves, none of these were found

to subtend buds, and the shoot dies down completely at the end of the season. The tuberous roots, on the other hand, may live for some few years, large specimens being fully three years old. In *Rhexia virginica* the seedling does not attain the flowering stage, and, as stated above, the stem withers completely at the end of the first season; consequently the vegetative reproduction by means of root-shoots plays a rôle of great importance. The seedlings of *Rh. mariana*, L., show the same structure, but the roots remain slender, and attain a great length in old specimens, ramify freely, and produce many shoots. Similar long and slender roots, developing shoots, were also observed in herbarium specimens of *Rhexia lutea*, Walt., *Rh. lanceolata*, Walt., *Rh. serrulata*, Nutt., and *Rh. ciliosa*, Michx.

Finally may be mentioned that root-shoots of the necessary type are characteristic of many Podostemaceae, of which the roots represent leaf-like or thread-like assimilating organs upon which vegetative shoots or flowers become developed (Warming).

BUDS UPON LEAVES.

The occurrence of buds upon leaves has been observed in some genera of various families, but, with the exception of the ferns, these buds are not so frequent as those upon roots. It would be a difficult matter, however, to classify them as ' reparative' or ' additional', since several cases are known where they develop upon the leaf of the same species naturally or artificially, the leaf being in a perfectly normal condition or injured, wounded, or cut off. Furthermore, the place where these buds appear is quite different, but seemingly constant in a number of cases, and the majority of the buds are developed upon the upper, the ventral, face of the leaf-blade.

Alexander Braun has given a list of plants developing buds upon the leaves, and he has arranged them according to the place where they occur: on the ventral face of the leaf-blade or of the petiole; on the margin of the leaf; on the dorsal face of the leaf; and, finally, on both faces. Several ferns, besides some Mono- and Dicotyledons, belong to the first category, with the buds developed on the ventral face. Among these are several species of *Asplenium, Aspidium, Phegopteris, Gymnogramme*, &c.; among the flowering plants bulblets have been observed in *Amorphophallus* and *Pinellia*; small shoots in *Nymphaea, Drosera, Chirita, Cardamine, Arabis*, &c. In *Malaxis, Bryophyllum*, and several ferns: *Ceratopteris thalictroides*, Brongn., *Aspidium vestitum*, Sw., and *A. proliferum*, R. Br., the buds appear along the margin. Only a very few cases are known where the buds appear on the dorsal face: *Cystopteris bulbifera, Asplenium celtidifolium*, and *Woodwardia radicans; Ornithogalum thyrsoides* is the only plant where buds (bulblets) have been observed on both faces, according to Turpin.

Several other cases have been described; for instance, *Tellima grandi-flora*, Dougl., which Douglas observed to produce young plants from the base of the leaf-blade; *Ranunculus bulbosus*, L., which sometimes develops bulblets upon the leaf, according to Dutrochet; the Tomato, from which Duchartre has described leaves, producing buds, growing out into branches; the multiplication of *Begonia* by means of 'leaf-cuttings' is well known, and finally may be added the singular vegetative reproduction shown by *Dentaria tenella*, Pursh, where several clusters of small tubers are developed upon the upper face of the petiole.

These examples represent a rather heterogeneous material, and several have actually only one feature in common, that they are regenerative buds developed upon leaves. We know that several of these buds appear only when the leaf is wounded or cut off (*Begonia, Achimenes, Ficus elastica, Bryophyllum, Drosera*), while others develop upon the leaf without any previous injury (the ferns, *Tellima, Chirita, Cardamine, Malaxis, Pinellia*, &c.). Moreover, we have seen that these buds may simply develop as small shoots with typical leaves (*Drosera, Tellima, Chirita, Begonia, Bryophyllum*, &c.), or they may develop in the form of bulblets (*Ranunculus, Malaxis, Ornithogalum, Pinellia*), or as small tubers (*Dentaria*). Biologically and morphologically these buds are thus very distinct. We know of no case, where they have developed new plants from leaves of ferns, which had been separated from the mother-plant. We have learned from the writings of Sachs and Goebel that the leaves of *Begonia* and *Achimenes* produce different buds according to the condition of the mother-plant. Thus by using leaf-cuttings of *Begonia*, taken from flowering specimens, the adventitious shoots will bloom earlier than if the mother-plant is not fully matured. Regarding *Achimenes*, leaves taken from the floral region of the stem will develop shoots blooming earlier than those taken from the basal, vegetative part of the plant. It is also a point of importance to notice the constant development of such regenerative buds in certain genera. Apart from the groups of ferns, where such cases are frequent, we have among the flowering plants *Malaxis, Pinellia, Bryophyllum, Dentaria*, and evidently also others, cases where these buds actually represent a part of the organization of the plant, since their fundaments can be traced in the embryonic condition of the foliar tissues. Buds of that kind are certainly distinct from most of the others, which may be frequent under certain conditions, and serve for the same purpose, 'vegetative reproduction', but they are evidently not homologous formations. They may, similarly to the root-shoots, represent 'reparative' or 'additional' formations, but several of these are not known sufficiently well for enabling us to establish such classification. Those of the ferns are undoubtedly all of the additional type; so also those of *Malaxis, Pinellia*, and *Dentaria*. But regarding *Bryophyllum* it is difficult to decide whether the buds are reparative or additional, since they are only

produced when the leaves have been removed—by the wind, for instance
—but their production is a constant character. In *Malaxis, Pinellia*, and
Dentaria the buds appear upon the leaves while still connected with
the mother-plant, and they develop new individuals when the foliage
has withered.

But whatever type these buds may be referred to in general, reparative
or additional, their function is the same—they all are regenerative. Their
geographical distribution shows that they are most frequent in the warm
climate, notably the tropics, and especially with regard to the ferns. In the
cold temperate zone some few flowering plants possess this power to pro-
duce buds upon the leaves; for instance, *Cardamine pratensis, C. amara,
Drosera rotundifolia, D. intermedia*, and *Malaxis*. In the arctic region
Cardamine pratensis produces such buds, though apparently seldom. The
species in which leaf-buds are developed are all perennials, and mostly
herbs. There does not seem to be any correlation between the development
of leaf-buds and the structure of the rhizome or any particular habit
of the plant. It was stated under the description of root-shoots that
these were seldom developed in stoloniferous plants. But characteristic of
both types of buds, those upon the leaves and those upon the roots, is the
constant repetition of the subterranean stem-structure possessed by the
mother-plant. In *Ranunculus bulbosus* and *Ornithogalum* the buds upon
the leaves develop into bulbils; in *Dentaria tenella* tubercles are produced
of the same structure as the rhizome; in *Drosera* the leaf-buds grow out in
exactly the same manner as the mother-plant; in *Scilla* the buds upon the
roots develop into bulblets, &c. Similarly corresponding structures recur
in the axillary buds of several herbs; we may refer to the axillary bulblets
in the inflorescence of *Saxifraga cernua* and *S. stellaris*, var. *comosa*; the
bulblets in *Dentaria bulbifera*; the tubercles in the axils of the upper leaves
of the so-called *Cicuta bulbifera*; the axillary bulblets in *Lilium bulbiferum*,
&c. Regenerative buds may thus occur as truly axillary upon aerial as
well as subterranean stems, and they may also occur as more or less
adventitious on roots and leaves; however, some of those which have been
observed upon leaves are not simply adventitious, but represent a part of
the organization of the individual—for instance, *Bryophyllum, Malaxis,
Pinellia*, &c.—and evidently to the same extent as the axillary bulblets and
tubercles of others. Vegetative reproduction is exemplified in very many
different forms, and those of the subterranean stems are of course the most
numerous and the most diversified. In the preceding pages we have
attempted to show that the production of buds upon roots and leaves is also
of some importance to the maintenance and distribution of the plant-
individual.

SUMMARY.

Many trees and shrubs of very different families develop root-shoots; only a very small number of herbs, about 180 species, possess that power. By Wittrock the root-shoots are classified as reparative, additional, and necessary, the additional being the most frequently occurring. Of these the reparative comprise such as are developed when roots become injured; the additional, on the other hand, on uninjured roots, and often in large numbers upon the same root; the necessary root-shoots may in some cases constitute a necessary part of the life-cycle of the plant; for instance, in *Cirsium arvense, Pyrola uniflora, Monotropa, Rhexia, Thladiantha*, &c. Some correlation exists between the development of root-shoots and the structure of the rhizome of the mother-plant; stoloniferous species, for instance, seldom produce root-shoots. Some exceptions have been recorded, however; for instance, *Pyrola, Chimaphila, Lupinus, Sium.*

Buds developed upon leaves is a much rarer occurrence, and most of the plants are ferns, especially tropical. The production of buds upon leaves is, in some cases, a constant character; thus it appears as if it really represents something more than an accidental or adventitious occurrence, as, for instance, in *Malaxis, Pinellia, Bryophyllum, Dentaria tenella.*

Common to both types of buds, on roots or leaves, is that they are regenerative, and that they develop into the same structural form of the mother-plant, viz. as a bulblet in *Ornithogalum, Scilla, Ranunculus bulbosus*, or as a tubercle in *Dentaria tenella*, as a leafy shoot in *Drosera, Chirita*, &c. They represent structures of no small importance to the individual, and constitute an interesting little chapter of plant-biology.

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NOTES.

ON A LABILE BLUE COMPOUND FROM THE POTATO TUBER.—In experiments carried out on potato juice, obtained in an atmosphere of carbon dioxide, colour changes were observed resembling those described by Haas and Hill in extracts of *Mercurialis perennis* ('Biochem. J.', 1925, xix. 236); when considered in relation to this work the observations made on the potato juice seem worth a brief note.

Slightly sprouted tubers (variety 'Abundance') were placed in a vacuum desiccator which was alternately evacuated and filled with carbon dioxide twice, and allowed to remain overnight. It was hoped that most of the oxygen present in the intercellular spaces might be removed in this way. On the following day the tubers were pulped in an atmosphere of carbon dioxide in an apparatus to be described shortly elsewhere.

The pulp, the microscopic examination of which showed that practically every cell had been broken up, fell on a filter-bed consisting of alternate layers of filter-paper pulp and sand, there being two layers of the former. The filtrate was faintly opalescent and of a beautiful light blue colour faintly tinged with green. When air was admitted very slowly and the juice agitated gently, the blue became more intense, though not as dark as the colour obtained by Haas and Hill from *Mercurialis perennis*. On further exposure to air the blue colour changed through various shades of green to a bright yellow. The yellow took on a reddish tinge and finally changed through the usual reddish-brown to brownish-black colours associated with the tyrosine-tyrosinase reaction. It was found that unless oxygen were admitted very slowly these latter colours in their rapid development tended to obscure the faint blue first obtained.

The experiment was repeated recently with another variety of potato ('King Edward'). The filtrate in this case was of a green colour, owing to the fact that the tubers used had been sprouted in the light ; nevertheless, on the cautious admission of air, the juice became bluer and darker. This extra blue shade of colour was discharged by the addition of a little sodium hydrosulphite, and reappeared on further admission of air, finally changing to a yellow-green colour.

When the blue-green solution was rendered faintly alkaline the blue was intensified. When it was treated with dilute mineral acids a salmon-pink colour was obtained. The results of all the above tests agree with those given by the substance from *Mercurialis perennis*.

On the other hand, boiling the blue-green solution with a saturated solution of ammonium sulphate did not give a carmine colour, as, according to a private communication, is the case with *Mercurialis perennis*.

These observations, incomplete as they are, suggest that the potato contains a compound resembling, or possibly generically related to, the one occurring in *Mercurialis perennis*. They are placed on record in this unfinished state because they appear to have some bearing on a question of physiological importance now being investigated by Haas and Hill, and the writer himself is unlikely to be able to follow them up in the near future.

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ON THE OCCURRENCE OF A DOUBLE LIGULE IN SELAGINELLA MARTENSII

—When examining a number of sections of the strobilus of *Selaginella Martensii*, which were prepared for class purposes from a plant growing in the Trinity College Botanic Gardens Dublin, I noticed in two cases that, in addition to the ordinary ligule, another smaller ligule was attached to a sporophyll.

Such an occurrence does not appear to have been recorded previously, and, in

Fig. 1   Fig. 2

view of our imperfect knowledge of the morphology and function of the ligule, any new facts with regard to this or its seem to be worthy of mention.

The two examples (Figs. 1 and 2) which are illustrated in the accompanying micro photographs were found in different cones from the same plant, and in each case only one sporophyll possessed the double ligule, the remaining ones on that cone being quite normal. The small additional ligule was situated close to the large one on the side more remote from the axis of the strobilus, and was quite distinct, being inserted on a separate base.

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